AQUAPORIN-1 SHIFTS THE CRITICAL TRANSMURAL PRESSURE TO COMPRESS THE AORTIC INTIMA AND CHANGE TRANSMURAL FLOW: THEORY AND IMPLICATIONS

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Abstract

Transmural-pressure ($\Delta P$)-driven plasma advection carries macromolecules into the vessel wall, the earliest pre-lesion atherosclerotic event. The wall’s hydraulic conductivity, $L_P$, the water flux to $\Delta P$ ratio, is high at low pressures, rapidly decreases and remains flat to high pressures (30, 1, 23, 19) due to pressure-induced subendothelial intima (SI) compression that causes endothelial cells to partially block internal elastic laminar fenestrae.

Nguyen et al. (19) showed that rat and bovine aortic endothelial cells express the membrane protein aquaporin-1 (AQP1) and transmural water transport is both transcellular and paracellular. They found that $L_P$ lowering by AQP1 blocking was perplexingly $\Delta P$-dependent. We hypothesize that AQP1 blocking lowers average SI pressure; therefore a lower $\Delta P$ achieves the critical force/area on the endothelium to partially block fenestrae. To test this hypothesis, we improve Huang et al.’s (10) approximate model and extend it by including transcellular AQP1 water flow. Results confirm observation in (19): wall $L_P$ and water transport decrease with AQP1 disabling. It predicts (1) low-pressure $L_P$ experiments correctly; (2) AQP1s contribute 30-40% to both the phenomenological endothelial+SI and intrinsic endothelial $L_P$; (3) the force on the endothelium for partial SI decompression with functioning AQP1s at 60 mmHg equals that on the endothelium at $\sim$43 mmHg with inactive AQP1s; (4) increasing endothelial AQP1 expression increases wall $L_P$ and shifts the $\Delta P$ regime where $L_P$ drops significantly to higher $\Delta P$. Thus AQP1 up-regulation (elevated wall $L_P$) might dilute and slow low density lipoprotein binding to SI extracellular matrix, which may be beneficial for early atherogenesis.

Keywords: wall hydraulic conductivity, Aquaporin-1, transcellular transport, atherosclerosis

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**New and Noteworthy:** Aortic endothelial cell aquaporin-1 participates in transmural-pressure-driven water flow. This flow may affect the kinetics of lipid-vessel wall matrix binding that can trigger pre-atherosclerotic lesions. Theory explains how aquaporin-1 expression changes alter vessel wall properties (intimal compression) to lower or enhance this flow, suggesting potential interventions to slow atherogenesis.

1 INTRODUCTION

Atherosclerosis begins when transmural pressure, $\Delta P$, driven advection transports (38, 9) macromolecules, e.g., low-density lipoprotein (LDL) cholesterol ($\sim$ 20-25 nm), from the lumen across focal leaks - rare (in rat $\sim$1 in 2000-6000 (15, 16, 14)) endothelial cells (EC) with wide (enough) junctions (34) - into the subendothelial intima (SI), where it spreads them radially from the leak. LDL reacts with SI extracellular matrix (ECM) to trigger a cascade of events leading to observable lesions. Since water, plasma’s major constituent, easily passes through the vastly more abundant normal EC tight junctions, $\Delta P$ drives far more water, albeit absent LDL, through normal than leaky junctions. This flow transports and dilutes SI LDL, potentially slowing LDL-ECM binding reaction kinetics and flushing unbound LDL from the SI and ultimately the wall. Starling’s law gives the overall transwall water flux, $J_w$, as (25): $J_w = L_P(\Delta P - \sigma \Delta \pi)$, with $L_P$ the vessel wall hydraulic conductivity, $\sigma$ the osmotic reflection coefficient and $\Delta \pi$ the transwall osmotic pressure difference. Since large vessels are generally considered isotonic (11) $^1$, one neglects $\Delta \pi$ and $J_w = L_P \times \Delta P$. Several groups (30, 1), including ours (23, 19), have measured $L_P$ of rabbit and rat aorta *ex vivo* over a range of $\Delta P$ and found $L_P$ for a vessel with intact endothelium is high at low $\Delta P$, decreases with increasing $\Delta P$ and then remains essentially flat to very high $\Delta P$. The same vessel’s deendothelialized $L_P$ is $\Delta P$-insensitive at $\sim$double its high-$\Delta P$ intact value. Ultrastructure studies (6) evince stark SI-media contrasts: a far sparser SI ($\sim$95% SI void (10)) vs media ECM (<50% void (9)). Huang et al. (10) thus suggested and later experimentally confirmed (8) that high $\Delta P$ compresses the SI. This causes ECs to partially block internal elastic laminar (IEL) fenestral pores, which inhibits water flow and decreases $L_P$. Once the proteoglycans (PG) are fully compressed, stiffer collagen (CG) fibers resist further compression ($L_P$ drop). Endothelial removal eliminates a resistance layer and fenestral blocking, making $L_P$ $\Delta P$-insensitive. This paracellular flow theory (10) agrees with all experimental $L_P(\Delta P)$ (30, 1) measurements.

Aquaporin-1 (AQP1), a highly water-specific channel protein in otherwise hydrophobic membranes found in various EC, epithelial and other cells (20), allows high $\Delta \pi$-driven water

$^1$A perfusate albumin concentration $>1$ g/dl (experiments in (19) use 4 g/dl) saturates surface glycocalyx and concentration polarization (CP), which can affect $L_P$ measurements, is weak (29). Since Ref. (19)’s filtration rates are within a factor of two (mostly far less) of each other, CP may slightly affect absolute, but hardly relative post-to-pre-HgCl$_2$ $L_P$ values.
throughputs (~3 \times 10^9 \text{ molecules/sec/channel}) at little or no ATP cost (18). Using immunohistochemical techniques, Nguyen et al. (19) showed AQP1 expression and distribution in rat (R) and bovine aortic (B)(A)ECs both in cultured monolayers and in whole rat aortas \textit{ex vivo}, suggesting possible transEC AQP1 water transport. They lowered functioning AQP1 numbers using very low HgCl_2 exposures titrated to non-toxic, reversible levels that chemically block AQP1s and repeated these experiments using siRNA against AQP1, both \textit{in vitro} (19) and, in a much more challenging procedure, on whole rat aortas \textit{ex vivo} (36). In all cases they found significant vessel wall and endothelial \(L_P\) reduction with reduced functioning AQP1 (\(L_P\) dropped 22.1±6% in BAECs with HgCl_2 and 56.4±7.9% in RAECs with siRNA, both vs control that indicated transEC flow). Studies with tracers that only cross the endothelium paracellularly show that these treatments cause insignificant junctional transport changes.

The \textit{ex vivo} studies measured \(L_P(\Delta P)\) of an excised vessel with functioning AQP1s, then with HgCl_2-blocked or knocked-down AQP1s, and then with the endothelium denuded, all on the same vessel. \(L_P\) dropped ~ 32±4%, 11±2%, 5±3%, for blocked AQP1s, at 60,100,140 mmHg respectively (19); they dropped 37±13.0% at 60 and 8.7±3.8% at 100 mmHg for the siRNA studies (36). The wall’s total resistance to flow, 1/\(L_P\), is the sum of the resistance of the endothelium+SI and that of the media+IEL in series, and the latter is \(\Delta P\)-insensitive. Since all \(L_P\) measurements were taken on each vessel, one can calculate the drops in each constituent \(L_P\) for each vessel and average. The percentage decreases in total wall \(L_P\) is then due to drops in endothelial+SI \(L_P\) of 51±2.3%, 21±3.6% and 11±6.5% at these three pressures. Clearly AQP1-mediated transcellular flow contributes significantly to endothelial \(L_P\). However it is confounding that this percent decrease is apparently strongly \(\Delta P\)-dependent, unlike for a simple material. Whereas \(L_P\) in the absence of blocker is pressure-dependent from 60 to 100 mmHg, when the AQP1s are blocked, \(L_P\) seems \(\Delta P\)-independent at these pressures. Reasoning from Huang et al. (10), we hypothesize that, whereas in the absence of blocker, the SI reaches full compression between 80 and 100 mmHg, with AQP1-blocking or knockdown, the SI fully compresses at 60 mmHg or less.

To test this hypothesis, this paper extends Huang et al.’s (10) local filtration theory by including transcellular flow. It hypothesizes that decreasing the number of functioning EC AQP1s decreases the number of available water transport pathways and thus the intrinsic endothelial hydraulic conductivity \(L_{P_e}\). A lower \(L_{P_e}\) decreases SI pressure \((P^*_i)\) at fixed \(\Delta P\), i.e., it increases the force per unit area, the difference, \((P^*_L - P^*_i)\), between lumen and SI pressures, acting on the endothelium. A lower overall \(\Delta P\) can thus compress the SI and cause partial fenestral blockage. We shall see if the theory explains Nguyen et al.’s (19) observed AQP-blocking effect on \(L_P(\Delta P)\).

Section 2 presents Huang et al.’s (10) filtration model and infinite series solution for the SI and media pressures using their three suggested approximate matching schemes in the IEL fenestrae and compares with a new finite difference implementation with exact matching there. Section
3 extends Huang et al.’s (10) model by incorporating transcellular flow. Section 3.3 compares the new model’s predicted $L_P(\Delta P)$ with experiment (19) and makes several predictions for future experiments, including those that may have clinical relevance.

2 FILTRATION MODEL: PARACELLULAR FLOW

2.1 Model Description

Since macromolecular transport across the artery wall is advection-dominated (9), it is critical to understand paracellular junctional (section 2) and transEC AQP1 (section 3) water flow in detail. 

$\Delta P$, the pressure difference between the inside (lumen) and outside (adventitia) of the vessel, drives water through the glycocalyx (GX) layer on the luminal EC surface into the SI through the interEC junctions along the EC perimeter. It then spreads radially parallel to the endothelium in the SI and enters the media through the IEL fenestrae (Figure 1). SI compression at increased $\Delta P$ narrows the cross-section for this SI flow and causes the endothelium to (partially) block the IEL fenestral entrance (10). The resulting decreased SI permeability, $K_P$, and this alteration in flow can lead to significant SI pressure gradients that can radically change the head losses incurred in traversing the SI and fenestrae. To understand the effect of SI compaction on these flows, we consider a local (on the scale of a single EC) model for SI flow into and through the fenestral hole. The model differs from Huang et al. (10) in that it also models the EC surface GX layer and treats the fenestral flow exactly.

By abuse of geometry (circles do not tile the plane), Figure 1 shows a representative local periodic wall unit of a circular cylinder of radius $\xi^* I=(R^*+\Delta R^*/2)$, where $R^*$ is the radius of an assumed round EC and $\Delta R^*$ is the width of a normal intercellular junction. The fluid source along the wall unit’s perimeter represents the normal EC junction. Since normal junctions vastly outnumber leaky junctions by a factor of 2000-6000 (15, 16, 14), they account for the overwhelming majority of water flow across the endothelium; this section thus models a cell with normal tight junctions. Figure 1 greatly exaggerates the vertical scale of the SI, which is of the order of 0.2-0.5 $\mu$m (9, 10) in healthy rat aorta. Experimental data (21) indicate that the number of fenestrae per EC is between 0.1 and 10; we take an average value of one fenestra as in (10). Thus a round fenestra of radius $r_f^*$ is ideally placed at the unit’s center concentric with the EC, an obvious idealization that preserves axisymmetry. Pressure loading acts on the assumed non-deformable endothelium to compress the SI from $L_i^* (\text{initial thickness at zero transmural pressure})$ to $L_i^*$. The model treats the IEL as an impenetrable barrier of zero thickness except for its fenestral openings and neglects any non-uniform deformation of the endothelium due to spatial differences in the transendothelial pressure. Due to the media’s high density, the model presumes that it undergoes no compression.
upon pressure loadings and assumes its filtration properties (e.g., $K_P$) are uniform.

### 2.2 Mathematical formulation

Let $j$ be a dummy index that takes values $g$ for GX, $i$ for SI and $m$ for media. Let $U_j^*, W_j^*$ be the dimensional lateral and normal velocities in the $r$ and $z$ directions of a cylindrical coordinate system and $P_j^*$, $K_P$ be the pressure and Darcy permeability, in region $j$ of thickness $L_j^*$. $\mu$ is the fluid viscosity. We neglect pressure pulsatility; thus let the time-invariant lumen pressure be $P_L^*$. Introduce the following non-dimensional (no superscript *) variables:

$$
\begin{align*}
    r &= \frac{r^*}{r_f^*}, \\
    \xi &= \frac{\xi_I}{r_f^*}, \\
    z_j &= \frac{z_j^*}{L_j^*}, \\
    P_j &= \frac{P_j^*}{P_L^*}, \\
    U_j &= \frac{U_j^*}{K_{P_j} P_L^* \mu}, \\
    W_j &= \frac{W_j^*}{K_{P_j} P_L^* \mu}, \\
    h_j &= \frac{L_j^*}{r_f^*}
\end{align*}
$$

The continuity equations, in non-dimensional form, for the three regions are:

$$
\begin{equation}
    h_j^2 \left( \frac{\partial U_j}{\partial r} + \frac{U_j}{r} \right) + \frac{\partial W_j}{\partial z_j} = 0 \quad (j = g, i, m)
\end{equation}
$$

where $h_j = \frac{L_j^*}{r_f^*}$ are the region thicknesses nondimensionalized by the fenestral radius. Porous media flow describes the water flow across the arterial wall: through the SI, made up of ECM of PG and CG fibers, and the media, consisting of smooth muscle cells (SMC), ECM and elastic layers. Darcy’s law: $V^* = \frac{-K_P}{\mu} \nabla P^*$ with an effective Darcy permeability for each region governs such flows. We use Darcy’s, rather than Brinkman’s equation because it does not seem consistent to explicitly enforce no slip on the region boundaries while simultaneously lumping the no-slip on the far more ubiquitous fibers, elastic layers and cells into the bulk parameters $K_P$. (A detailed analysis of this issue in such problems shows only a minor effect (9)) Thus, Eqs. 1 become:

$$
\begin{equation}
    h_j^2 \left( \frac{\partial^2 P_j}{\partial r^2} + \frac{1}{r} \frac{\partial P_j}{\partial r} \right) + \frac{\partial^2 P_j}{\partial z_j^2} = 0 \quad (j = g, i, m)
\end{equation}
$$

The (non-dimensional) boundary conditions for this system of coupled partial differential equations (PDEs) follow: (Note $z_m^* = 0$ is at the IEL, not at the EC, both modeled as infinitely thin.)

(a) Axisymmetry at $r = 0$ and periodicity (and, therefore, no radial flux) at $r = \xi_I$ require:

$$
\frac{dP_j}{dr} = 0 \text{ at } r = 0 \text{ and } r = \xi_I \quad (j = g, i, m)
$$

(3)
(b) The pressure at the top of the GX layer equals the lumen pressure:

\[ P_g = 1 \text{ at } z_g = 1 \]  \hspace{1cm} (4)

(c) The adventitia is assumed to be at the reference pressure:

\[ P_m = 0 \text{ at } z_m = -1 \]  \hspace{1cm} (5)

(d) Mixed boundary conditions at the GX/EC boundary \((z_i = 1)\): The endothelium is assumed impermeable to water (relaxed in section 3). The hydraulic conductivity of the normal junction \((L_{Pnj})\) and the pressure difference across it govern the amount of water entering the SI through it.

(d1) On the EC: \((0 < r \leq R, z_i = 1)\)

\[ \frac{dP_j}{dz_j} = 0 \hspace{1cm} (j = g, i) \]  \hspace{1cm} (6)

(d2) In the normal junction: \((R < r \leq \xi_I, z_i = 1)\)

\[ W_g = W_i \]  \hspace{1cm} (7)

\[ -W_i = \frac{L_{Pnj} \mu L^*_i (P_g - P_i)}{K_{P_i}} \]  \hspace{1cm} (8)

Previous studies (39) that modeled the vessel wall over radial scales equivalent to 1000 EC radii used \(L_{Pnj}\) to describe the area-averaged hydraulic conductivity of the endothelium (ECs and normal junctions), equivalent to \(L_{Pe}\) in this local model. Here we allow only junctional water transport; so \(L_{Pe}\) and our \(L_{Pnj}\) are related by the ratio of junctional to total endothelial areas; section 3 includes a separate transcellular contribution, and both will contribute to an area averaged \(L_{Pe}\).

(e) Mixed boundary conditions at the SI/media boundary \((z_i = 0)\): Assume the IEL is an impenetrable barrier except for its fenestral openings. Thus, water enters the media only through the fenestra where pressures and velocities are continuous. This requires

(e1) In the fenstral hole: \((0 < r \leq 1, z_i = 0)\)

\[ P_i = P_m \]  \hspace{1cm} (9)

\[ W_i = W_m. \]  \hspace{1cm} (10)
(e1) Outside the fenstral hole: \( 1 < r \leq \xi_I, z_i = 0 \)

\[
\frac{dP_j}{dz_j} = 0 \quad (j = i, m).
\]

(11)

Huang et al.’s model (10) omitted the GX layer on the EC’s luminal side. Instead of periodicity at \( r = \xi_I \) in the SI (Eq. 3), they assumed a ring source at the EC cleft and determined the unknown constant pressure \( P_0 \) at \( r = \xi_I, 0 \leq z \leq 1 \) by imposing flow incompressibility. With these simplifications, Huang et al. (10) found analytical solutions of Eq. 2 for the SI and media pressures by decomposing the pressures into orthogonal pieces in \( r \), solving the \( z \)-dependence of each piece and reassembling the infinite sums of zero order Bessel functions \( (J_0) \) (graphed in Fig. 2):

\[
P_i(r, z_i) = P_0 + P_0 \sum_{n=1}^{\infty} \left( A_n \frac{\cosh[h_i \sqrt{\lambda_n} (z_i - 1)]}{\cosh(h_i \sqrt{\lambda_n})} \cdot J_0(\sqrt{\lambda_n} r) \right),
\]

(12)

\[
P_m(r, z_m) = P_0(z_m + 1)C_0 + P_0 \sum_{p=1}^{\infty} \left( C_p \frac{\sinh[h_m \sqrt{\lambda_p} (z_m + 1)]}{\sinh(h_m \sqrt{\lambda_p})} \cdot J_0(\sqrt{\lambda_p} r) \right),
\]

(13)

where \( \lambda_n \) and \( \lambda_p \) are the roots of the eigenvalue equations \( J_0(\sqrt{\lambda_n} \xi_I) = 0 \) \( (n = 1, 2, 3, \ldots, \infty) \) and \( J_1(\sqrt{\lambda_p} \xi_I) = 0 \) \( (p = 1, 2, 3, \ldots, \infty) \). The constants \( A_n \) and \( C_p \) depend on the fenestral boundary conditions, Eqs. 9, 10. Huang et al. (10) approximated these fenestral \( (0 < r \leq 1, z_i = 0) \) conditions in the following three ways and Fig. 2 will show their effect on the pressure distribution:

(i) The \( z \)-velocity, \( W_f \), in the fenestra is uniform on \( 0 < r \leq 1, z_i = 0 \) and the pressures in the SI and media exactly match only at the fenestra center i.e., \( P_i = P_m \) at \( r=0, z_i = 0 \);

(ii) \( W_f \) is uniform on \( 0 < r \leq 1, z_i = 0 \) and only the average pressure, \( \bar{P}_j = 2 \int_0^1 P_j(r) r dr, j = i, m \), across the fenestra matches in the fenestral hole i.e., \( P_i = P_m \) at \( z_i = 0 \);

(iii) At \( z = 0 \), \( W_f(r) \) fits a cubic polynomial that satisfies \( dW_f/dr = 0 \) at \( r=0 \) and the pressure is continuous only at three points in the fenestra, \( r = 0, 0.5, 0.9 \) i.e.,

\[
W_f = (a_0 + c_0 r^2 + d_0 r^3)P_0 \quad \text{and} \quad P_i = P_m \quad \text{at} \quad r = 0, 0.5, 0.9; \quad z = 0.
\]

2.3 Exact numerical solution of the boundary value problem:

We adopt a direct-discretization, finite difference approach using central difference formulae for non-uniform meshing (22) to solve the system of coupled PDEs, Eq. 2. Though the governing
equations for the filtration problem are Laplace equations, the mixed boundary conditions (Eqs. 3-11) make it difficult to obtain an exact analytical solution. This numerical method, in principle, allows us to use the exact boundary conditions rather than the approximate ones employed by Huang et al. (10). Since the thickness of the SI ($L_{si}^*$) and the radius of the fenestral hole ($r_{f}^*$) are both small compared to both the radius of wall unit ($\xi_j^*$) and the length of media ($L_{m}^*$), we use non-uniform grids in the $r$ and $z$ directions. We expect a steep pressure gradient near the fenestral hole and thus form a very dense grid there with the smallest non-dimensional grid size ($r^*/r_f^*$) of 0.0005 (non-dimensional $r$, $z_p$, $z_i$ and $z_m$ vary from 0 to 15.0125, 0 to 1, 0 to 1 and 0 to -1, respectively) in the fenestral hole. To resolve the pressure variations in the normal junction (width $\sim 20$ nm (10)), we use an extremely fine grid near the junction with the smallest grid size being $8 \times 10^{-10}$. Since the coefficients ($L_{i}^*$, $L_{m}^*$, $K_{P_i}$, $K_{P_m}$) in the velocity matching condition (Eq. 10) differ by several orders of magnitude between regions being matched (see Sec. 2.2), one has to be very careful in selecting the mesh sizes in the $z$-directions near the fenestral hole. If the mesh size is not sufficiently small, the finite difference approximations used for the derivative $dP/dz$ could lead to significant errors there. We chose the smallest grid size near the hole to be 0.0005 (SI) and 0.000001 (media). Similarly, the smallest grid sizes used in the $z$-directions close to the endothelial cell boundary are 0.0005 and 0.000006 in the SI and GX regions.

We use second order difference formulae to discretize the boundary conditions. This leads to a linear system of algebraic equations whose number equals the total number of mesh points. We solve the set of equations representing the three domains simultaneously using Matlab (Mathworks Inc). To test the accuracy of the solution, we adopt a successive mesh-refinement procedure until the difference between two consecutive computations is in the fourth significant digit.

2.4 Constants and Parameters

2.4.1 Geometric parameters:

Most of the parameters used in this study, given in table 3, are adopted from Huang et al. (10). They extracted an average spacing, $\delta_{PG}$, between PG fibers of approximately 30-40 nm from Frank and Fogelman’s (6) freeze etchings. We use $\delta_{PG0} = 40$ nm for the relaxed SI. Huang et al. (9) developed a fiber matrix theory to calculate the effective radius ($a^*$) of the PGs and the zero $\Delta P$ void fraction ($\epsilon_{PG0}$) for the PGs. We use Huang et al.’s (9) theory to estimate $a^* \sim 2.37$ nm and $\epsilon_{PG0} \sim 98.83\%$. Using these values, we calculate the Darcy permeability of a fiber matrix of PGs in the SI using the Carman-Kozeny expression (5, 2, 3) as:

$$K_{P(PG)} = \frac{a^2 \epsilon_{PG0}^3}{4G(1 - \epsilon_{PG0}^2)}$$
where $G$, the Kozeny constant, is obtained as in (7). The spacing, $\delta_{CG0}$, between radius 20 nm (9) CG fibers, assumed to form a parallel, triangular fiber array with a zero-$\Delta P$ volume fraction, $\varepsilon_{CG0} \sim 5\%$ (9), is calculated as 170.35 nm. Tsay and Weinbaum’s correlation (31) gives the collagen matrix Darcy permeability, $K_{P(CG)}$, in terms of the average fiber radius and spacing:

$$\frac{K_{P(PG)}}{K_{P(CG)}} = \left(\frac{r_{CG}^*}{a^*}\right)^{0.377} \left(\frac{\delta_{PG0} - 2a^*}{\delta_{CG0} - 2r_{CG}^*}\right)^{2.377}.$$  

We assume that the resistances due to the PG and CG fiber populations act as resistors in series and thus compute the overall SI Darcy permeability, ($K_P$), as (13):

$$\frac{1}{K_P} = \frac{1}{K_{P(PG)}} + \frac{1}{K_{P(CG)}}.$$  

At zero $\Delta P$, we calculate $K_P = 2.20 \times 10^{-12}$ cm$^2$, in agreement with Huang et al. (10). As in Huang et al. (8), $\Delta P$-induced SI compression alters the void fractions for the PG ($\varepsilon_{PG}$) and CG fibers ($\varepsilon_{CG}$) via the SI thickness, $L_i^*$, as:

$$\varepsilon_{PG} = 1 - \frac{L_i^*}{L_{i0}^*} (1 - \varepsilon_{PG0});$$

$$\varepsilon_{CG} = 1 - \frac{L_i^*}{L_{i0}^*} (1 - \varepsilon_{CG0}).$$

At a given compression, one calculates the PG and CG void fractions and estimates the average spacing between these fibers assuming they form a parallel, triangular array. With this spacing, we compute $K_P$ of the compressed SI as explained. As in Huang et al. (10), we find that $K_P$ decreases rapidly as the SI starts compressing with pressure loading. $K_P$ at $L_i^* = 0.2L_{i0}^*$ ($L_i^* = 0.1L_{i0}^*$) is $2.06 \times 10^{-13}$ cm$^2$ ($4.7 \times 10^{-14}$ cm$^2$), an order (approximately two orders) of magnitude lower than its uncompressed value. This drastic drop in $K_P$ significantly affects the pressure and velocity distributions in the vicinity of the fenestra, as explained in the results.

The major constituents of the GX layer are PGs, which include both glycosaminoglycan side chains and glycoproteins (28). Recent observations of Squire et al. (24) and Weinbaum et al. (33) suggest a bush-like GX structure with clusters of core proteins projecting normally from the EC surface. We model the GX as a fiber matrix with Darcy permeability, $K_{P_g}$, defined as (31):

$$K_{P_g} = 0.0572 a_f^2 \left(\frac{\Delta}{a_f}\right)^{2.377},$$

where $a_f$ is the fiber radius (6 nm (24)) and $\Delta$ the open spacing between fibers (8 nm (24)),
giving \( K_{P_g} = 4.08 \times 10^{-14} \text{ cm}^2 \). Using \( \Delta = 20 \text{ nm} \), Dabagh et al. (4) calculated \( K_{P_g} \) as \( 3.6 \times 10^{-13} \text{ cm}^2 \). Assuming a regular 2-D hexagonal arrangement, we compute the fiber volume fraction to be 0.326 and thus a void fraction of 0.674. Using this void fraction and Zhang et al.’s (41) relation between fiber radius, void fraction and the open flow area between fibers, Liu et al. (17) estimated \( K_{P_g} \) as \( 6.04 \times 10^{-14} \text{ cm}^2 \). Seki et al. (26) calculated the GX Darcy permeability considering flows perpendicular \( (3.16 \times 10^{-14} \text{ cm}^2) \) and parallel \( (6.10 \times 10^{-14} \text{ cm}^2) \) to a hexagonal array of cylindrical fibers. Our \( K_{P_g} \) value lies in this range. We assume that, since the GX lies between the lumen and the EC, increasing lumen pressure affects neither the GX nor its structural properties.

2.4.2 Hydraulic conductivities:

As described in section 1, the engineering quantity, hydraulic conductivity, \( L_P \), the ratio of the fluid velocity to the driving pressure difference, is \( \Delta P \)-independent for simple materials. We are concerned with the \( L_P \)'s of the endothelium, the normal junction, SI, IEL, media, endothelium + SI, IEL + media (i.e., the denuded vessel), and total arterial wall, denoted as: \( L_{P_e}, L_{P_n}, L_{P_i}, L_{P_l}, L_{P_m}, L_{P_{e+i}}, L_{P_{m+I}}, L_{P_t} \), respectively. Tedgui & Lever (30) and Baldwin & Wilson (1) measured \( L_{P_t} \) and \( L_{P_{m+I}} \) for rabbit aorta over a range of \( \Delta P \)s, while Shou et al. (23) and Nguyen et al. (19) did analogous experiments on rat aortas. The latter three groups did measurements at all \( \Delta P \)s for the intact \( (L_{P_t}) \) and then for the denuded \( (L_{P_{m+I}}) \) aorta, all on each vessel. One can calculate the phenomenological \( L_{P_{e+i}} \) for each vessel. \( (1/L_P) \) is a specific resistance. Since linear resistances in series add, the total resistance is related to the layer resistances by:

\[
\frac{1}{L_{P_t}} = \frac{1}{L_{P_{e+i}}} + \frac{1}{L_{P_{m+I}}} \quad (14a)
\]

\[
\frac{1}{L_{P_{e+i}}} = \frac{1}{L_{P_e}} + \frac{1}{L_{P_i}} \quad (14b)
\]

\[
\frac{1}{L_{P_{m+I}}} = \frac{1}{L_{P_m}} + \frac{1}{L_{P_I}} \quad (14c)
\]

\[
K_{P_m} = L_{P_m} \times \mu \times L_m^*, \text{ where } \mu \text{ is the viscosity of water and } L_m^*, \text{ the media thickness, is } 125 \text{ \( \mu \text{m} \) (141 \( \mu \text{m} \)) for rabbit (rat) aorta. Note that the rat aorta media thickness measurement is larger because Shou et al. (23) measured it on vessels fixed after excision rather than on vessels fixed } \text{insitu}, \text{ where it is still tethered and in a stretched state inside the animal. Excision releases this stretching, causing the vessel to retract and, by mass conservation, become thicker. At steady state (for fixed geometry, even in the unsteady case), fluid incompressibility requires the water flow across each arterial layer to be the same. Thus, as explained by Huang et al. (10), by matching water fluxes across the IEL and using Eqs. 20 and 21 from Ref. (10) with the average } L_{P_{m+I}} \text{ for}
each data set given in Table 1, we obtain $L_{P_m} = 11 \times 10^{-8}$ cm/sec · mmHg for Tedgui & Lever’s data (30). This gives us $K_{P_m}$ as $8.38 \times 10^{-15}$ cm², which is very close to $6.09 \times 10^{-15}$ cm² used by Tada et al. (27). Table 4 lists the calculated values of $L_{P_m}$ for the other data sets.

The by-nature pressure-independent intrinsic hydraulic conductivity of the endothelium, $L_{P_e}$, is unknown. We assume that at very low $\Delta P$ the SI is fully expanded and there is no fenestral blocking. So the combination of SI $K_P$ and the squeezing flow into the IEL fenestra should only account for a small part of the total resistance. (Eq. 14b guarantees $L_{P_e} \geq L_{P_{e+i}|\Delta P(min)}$.) Thus, one can initially estimate $L_{P_e}$ by $L_{P_{e+i}}$ for the least compressed SI configuration for which $L_{P_t}$ data are available. One uses this value to solve the flow problem subject to boundary conditions Eqs. 3-11 and then calculates $L_{P{e+i}}$ from flow incompressibility, i.e., the overall water flow across the vessel wall must match the water flow across the endothelium as well as across the IEL:

$$L_{P_{e+i}}(\bar{P}_g|z_g=0 - \bar{P}_i|z_i=0) \pi\xi^2_I = \frac{2\pi K_{P_i}}{\mu L^*_i} \int_0^1 \frac{dP_i}{dz_i}|_{z_i=0} r dr$$  \hspace{1cm} (15)

where,

$$\bar{P}_g|z_g=0 = \frac{2}{\xi^2_I} \int_0^{\xi_I} P_g|z_g=0 r dr \quad \text{and} \quad \bar{P}_i|z_i=0 = \frac{2}{\xi^2_I} \int_0^{\xi_I} P_i|z_i=0 r dr$$  \hspace{1cm} (16)

If this $L_{P_{e+i}}$ does not match the experimental value in Table 1 with which we started, we adjust $L_{P_e}$ and iterate until the two $L_{P_{e+i}}$ values match. This approach is only slightly more complicated than that in (10) due to the GX layer here. $L_{P_e}$ is the ratio of junction to total area times $L_{P_{nj}}$:

$$L_{P_e} = \frac{L_{P_{nj}} 2\pi R^* (\Delta R^*/2)}{\pi\xi^2_I}.$$  \hspace{1cm} (17)

Table 5 gives the converged results for $L_{P_e}$ for the available data sets. We use these converged values of $L_{P_e}$ (or, in this section, $L_{P_{nj}}$) for all further calculations and predictions.

One test of the model is to compare the computed and experimental total wall $L_{P_t}$ data. One way to find $L_{P_t}$ is to insert $L_{P_{e+i}}$ from Eq. 15 and an averaged $L_{P_{m+i}}$ from the corresponding experimental data into Eq. 14a, as done in (10). However, $L_{P_{m+i}}$ only enters the model calculation in determining $K_{P_m}$. The calculated media pressure field at each $\Delta P$ leads to a phenomenological $L_{P_{m+i}}$ for Eq. 14a via an equation analogous to Eq. 15. This $L_{P_{m+i}}$, the media+IEL conductivity in an intact wall, might neither equal the averaged experimental value used to find $K_{P_m}$ nor even be $\Delta P$-independent, as is $L_{P_{m+i}}$ for the denuded wall. In fact, $L_{P_{m+i}}$ calculated this way turns out to be nearly constant at low $\Delta P$, but decreases significantly at higher $\Delta P$. A better method for finding a fully model-generated value for $L_{P_t}$ is to use fluid incompressibility to equate the total
flow across the wall in terms of $L_{P_i}$ with the calculated flow through the fenestral hole:

$$L_{P_i} \pi \xi_i^2 = \frac{2\pi K_{P_i}}{\mu L_i^*} \int_0^1 \left| \frac{dP_i}{dz_i} \right|_{z_i=0} rdr$$  \hspace{1cm} (17)

### 2.5 Results and discussion

#### 2.5.1 Pressure drop across the IEL:

Figures 2a and 2b depict the pressures, non-dimensionalized by the luminal pressure $P_L^*$ (the adventitial pressure is the zero reference pressure), above and below the IEL as a function of $r^*/r_f^*$ for various intimal compressions. Figure 2a uses the analytical series solution with Huang et al.'s (10) approximate boundary conditions (section 2.2) and Fig. 2b the finite difference solution with the exact boundary conditions. As in Huang et al. (10), Fig. 2a uses a ring source at the normal junction instead of Eqs. 7 and 8. Fig. 2b properly matches the pressure and velocity in the fenestral hole ($r^*/r_f^* \leq 1$) and includes the GX layer and its matching conditions, Eqs. 7 and 8. In both figures the pressure decreases from the edge of the wall unit to the center on the SI side as the fluid approaches the fenestral hole and then decreases as $r$ increases on the media side as the fluid exiting the fenestra spreads. Both sets of curves display a qualitative change in the pressure as the SI thins in response to increasing transmural pressure. For $L_i^* \geq 200$ nm, the pressure in the SI is almost flat; most of the pressure drop occurs in the media. As the SI thins with increasing $\Delta P$, say at $L_i^* \sim 50$ nm, most of the pressure drop shifts to the SI within several pore radii from the fenestral opening. Thus the flow resistance shifts with pressure from the medial spreading flow to the entrance flow into an IEL fenestra that is partially blocked by an EC and water incurs a sharp pressure drop (50nm curve in Fig. 2b) in traversing the SI.

Figure 2a is nearly identical to Huang et al.'s (10) with small differences likely due to different numbers of terms retained ($\sim 50$ in (10) vs. 200 here) and a different matrix inversion tool used. Figures 2a and 2b agree well except in and near the fenestral hole, especially for compressed intimae where the predictions are off by $\sim 10\%$ and in the junction region, where they differ by roughly 3%. These two regions are where the pressures vary significantly in $z$ and, for thinner SI, in $r$ across the fenestra or junction. Except in these regions, the characteristic $z$-scale, $L_{i0}$, is far smaller than the characteristic $r$-scale, $\xi_i$, i.e., the $z$ variation of the dynamic variables should be very small (not of leading order in $L_{i0}/\xi_i < < 1$). This scale separation fails and $z$-variation is significant in these regions, since the far smaller region width replaces $\xi_i$ as the $r$-scale. Huang et al.'s (10) third, three-point-matching approximation is closer to the exact solution than their other two schemes (see figure caption), as anticipated. To find the dimensional pressures, section 2.5.2 uses Huang et al.'s (10) theory to first predict the extent of SI compression for a given $P_L^*$. 

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2.5.2 Pressure dependent hydraulic conductivity:

To compare the predicted \( L_{Pt} \) with the four sets of measured \( L_{Pt}(\Delta P) \) for the intact arterial wall, we model the SI matrix as a Hookean spring. The spring responds linearly to the force per unit area, \( P^*_L(\bar{P}_g - \bar{P}_i) \), on the endothelium with a SI compression, \( L^*_i/L^*_{i0} \):

\[
P^*_L(\bar{P}_g - \bar{P}_i) = k \left(1 - \frac{L^*_i}{L^*_{i0}}\right), \tag{18}
\]

where \( k \) is the \( \Delta P \)-independent SI elastic coefficient (spring constant) [mmHg]. Using Eqs. 17 and 16, we calculate \( L_{Pt} \) and \( \bar{P}_g - \bar{P}_i \) at different SI thicknesses, \( L^*_i \). For each \( \Delta P \), we find the SI thickness for which the calculated \( L_{Pt} \) matches experiment. For these \( L_{Pt}, P^*_L \) and \( L^*_i \), the best fit slope of transendothelial pressure vs endothelial displacement (Eq. 18) is \( k \).

Figure 3 plots the SI thickness, \( L^*_i/L^*_{i0} \), vs lumen pressure, \( P^*_L \), from Eq. 18 using the \( k 's \) determined from four different \( L_{Pt}(\Delta P) \) data sets and all four curves compare well with (within the error bars of) Huang et al.’s (8) data for \( L^*_i/L^*_{i0} \) of rat aorta fixed at four different \( \Delta P \)s. Given the complexity of measuring the thickness of the extremely irregular SI layer and the simplicity of our model, the model seems to capture the important physiology that governs SI compression. SI thickness decreases nearly linearly with \( \Delta P \) for \( \Delta P (<\sim 60 \text{ mmHg} \) for Baldwin & Wilson’s (1) and Nguyen et al.’s (19) data and \( <\sim 80 \text{ mmHg} \) for Tedgui & Lever’s (30) and Shou et al.’s (23) data), implying the transendothelial pressure difference is essentially proportional to \( \Delta P \) there. Further increase in luminal pressure causes a nonlinear flattening of the \( L^*_i/L^*_{i0} \) vs \( \Delta P \) until a critical \( \Delta P \) beyond which no further compaction occurs. This explains why \( L_{Pt} \) becomes \( \Delta P \)-independent beyond this critical pressure. Our (Huang et al.’s (10)) model predicts the critical thickness \( L^*_{ic} \) (that corresponds to the critical pressure) relative to its unstressed value (\( L^*_{i0} \)) to be \( \sim 14\% \) (13\%) for Tedgui & Lever’s (30), 16\% (16\%) for Baldwin and Wilson’s (1), 16\% for Shou et al.’s (23) and 15\% for Nguyen et al.’s (19) data (the latter two sets postdate Huang et al. (10)).

Using Eq. 17, Fig. 4 plots the calculated \( L_{Pt} \) for the intact and average \( L_{P_{m+1}} \) for the denuded vessels as functions of \( \Delta P \), compares with each of the four different experimental data sets and fits a value of \( k \) for each data set. Since Shou et al.’s (23) rat and Tedgui and Lever’s (30) rabbit \( L_{Pt} \) and \( L_{P_{m+1}} \) data overlap, we assume the same \( L^*_{i0}=500 \text{ nm} \) for both rabbit and rat data. Agreement is good for all data sets. When the vessel is intact, \( L_{Pt} \) is nearly constant until \( \sim 60 \text{ mmHg} \), after which it drops nonlinearly by \( \sim 40\% \) until it reaches the critical \( \Delta P \) where the force/area on the endothelium reaches its critical limit for maximal SI compression. \( L_{Pt} \) remains constant for \( \Delta P \geq 100 \text{ mmHg} \). \( L_{P_{m+1}} \) of the denuded vessel is \( \Delta P \)-independent since there is no SI in the denuded vessel to compress and no ECs to block IEL fenestrae. As in Huang et al. (10), this theory explains the observed shapes of the \( L_{Pt} \) and \( L_{P_{m+1}} \) vs \( \Delta P \) curves, including the marked
drop in $L_P$ over a 60-100 mmHg dynamic pressure range.

As noted, unlike our use of Eq. 17 for $L_P$, Huang et al. (10) used their calculated pressure field to estimate $L_{Pc+i}$ (Eq. 10 in (10)) and, used it with a constant, data set-specific $L_{Pm+i}$ value in Eq. 14a to predict $L_P$ vs $\Delta P$. We find (not shown) their method slightly ($\sim$2%) underpredicts $L_P$ at low SI compressions, but overpredicts it (by 8-20% for $L_i^*/L_{i0}^* \sim 0.2-0.15$) at high compressions. Our method finds spring constants for Tegui and Lever (30), Shou et al. (23) and Nguyen et al. (19) all $\sim$30 mmHg (32.7, 27.66, 30.6 mmHg), but lower (23.68) for Baldwin and Wilson’s (1) rabbit data, whose $L_P$ are uniformly $\sim$ double the $L_P$s of the other rat and rabbit data sets. Huang et al.’s (10) method gave lower $k$s, 22(16) mmHg, for Tedgui & Lever (30) (Baldwin & Wilson’s (1)) data. Higher $k$ means a stiffer spring, i.e., a higher force/area on the endothelium is needed to achieve the same level of compression. Since both models predict roughly the same critical SI thickness, our predictions with higher $k$ correspond to a higher critical transendothelial pressure difference, which, given differing $L_{Pc}$,s, may or may not mean a higher $\Delta P$ to achieve maximal SI compression. Our predicted critical $\Delta P$s for Tedgui & Lever’s (30) (Baldwin & Wilson’s (1)) data (Fig. 4a and b) are 124 (96) mmHg, compared with Huang et al.’s (10) 135 (82) mmHg. We predict critical $\Delta P$s of 100 (90) mmHg for Shou et al.’s (23) (Nguyen et al.’s (19)) data.
3 FILTRATION MODEL: TRANSCELLULAR FLOW

Nguyen et al. (19) and Xue (36) observed marked decreases in $L_{Pe}$ in culture and in $L_{Pe}$ and $L_{Pe+i}$ in whole rat aortas ex vivo with AQP1 blocking or knockdown. They infer a significant functional role of AQP1-mediated transcellular flow in determining and controlling transmural water transport through rat arterial walls. What is confounding is the fact that their observed drops in $L_{Pe}$ with AQP1 blocking differ significantly at different $\Delta P$s. Huang et al.’s (10) SI compaction theory (section 2) yields a critical average force per unit area $(\bar{P}_g - \bar{P}_i^*)$ on the endothelium to compress the SI, which occurs with normal AQP1s over the dynamic $\Delta P$ range of $\sim 60-100$ mmHg. We propose that blocking AQP1 channels decreases the available pathways for water transport across the endothelium, thereby decreasing $L_{Pe}$ and consequently $P_i^*$. At fixed $\Delta P$, this increases the average force per unit area $(\bar{P}_g - \bar{P}_i^*)$ acting on the endothelium and shifts a larger fraction of $\Delta P$ from the media to the endothelium. The critical force per unit area acting on the endothelium thus obtains at a lower $\Delta P$, which should shift the dynamic range for SI compression to lower $\Delta P$. At high $\Delta P$, where (average) partial fenestral blocking takes place even at normal functioning AQP1 levels, reducing AQP1 function would only lower $L_{Pe}$, which should only minimally lower $L_{Pi}$, as Nguyen et al. (19) observed. To test this hypothesis, we incorporate transcellular flow into the model in section 2 and use it to quantitatively explain the contribution of AQP1s to the $\Delta P$-independent intrinsic endothelial $L_{Pe}$ and the $\Delta P$-dependent vessel wall $L_{Pi}$ and phenomenological $L_{Pe+i}$.

3.1 Mathematical formulation

Transcellular water flow alters only section 2’s endothelial ($z_i = 1$) boundary condition from EC impermeability to a non-zero EC hydraulic conductivity, $L_{PEC}$, due to AQP1. The endothelium’s intrinsic $L_{Pe}$ is now the area-average of $L_{PEC}$ and the junctional $L_{Pnj}$. The junctional boundary conditions, Eqs. 7 and 8, do not change, but on the EC ($z = 1; 0 < r \leq R$) Eq. 19 replaces Eq. 6:

$$-W_j = \frac{L_{PEC}\mu L_{i}^*(P_g - P_i)}{K_i} \text{ at } z_i = 1 \quad (j = g, i);$$  \hspace{1cm} (19)

$$L_{Pe} \pi \xi_i^2 = L_{PEC} \pi R^* + L_{Pnj} 2\pi R^*(\Delta R^*/2).$$  \hspace{1cm} (20)

Eq. 20 allocates half the junctional area to the cell in question and half, by symmetry, to its neighboring cells. With only this change, we solve Eq. 2 by finite difference exactly as in section 2.
3.2 Constants and parameters

The only new parameter not appearing in section 2.4 is \( L_{PEC} \). To determine the AQP1 fraction of \( L_{P_e} \), whose intrinsic value we calculated from experiment, we take \( L_{PEC} \) to be various fractions of \( L_{P_e} \). Eq. 20 gives the (now lower) \( L_{P_{nj}} \) corresponding to each assumed fraction.

3.3 Results and discussion

3.3.1 Pressure-dependent intact aorta hydraulic conductivity, \( L_{P_t} \), for unblocked AQP1s:

Functioning, unblocked AQP1s provide pathways, in parallel with the intercellular junctions, for substantial transendothelial water flow. To predict \( L_{P_t}(\Delta P) \), we fix \( L_{P_e} \), vary the fraction of \( L_{P_e} \) due to \( L_{PEC} \) and calculate the intact wall \( L_{P_t} \) for various SI thicknesses. For each \( L_{PEC} \) fraction, we use the procedure in section 2.5.2 to find the compression that gives \( L_{P_t} \) equal to the measured value in, e.g., Nguyen et al.’s (19) unblocked data, at that \( \Delta P \). The slope of the resulting compressions vs the transendothelial force/area curve is the SI ECM spring constant \((k)\); Eq. 18 gives the corresponding SI thickness at intermediate \( \Delta P \). With these inputs one solves the model for the pressure field, uses Eq. 17 to find \( L_{P_t} \) and plots \( L_{P_t} \) vs \( \Delta P \) for each assumed AQP1 fraction.

Figure 5 shows \( L_{P_t} \) vs \( \Delta P \) for various (open, functioning) AQP1 fractions of \( L_{P_e} \) at fixed \( k \). These curves all have the same \( L_{P_e} \), but different ratios of para-to-transcellular flow. Identical \( L_{P_{nj}} \) and \( L_{P_e} \) and experimental \( L_{P_t} \) force all curves to have the same low and high \( \Delta P \) plateaus. The SI streamlines (not shown) without AQPs are parallel to the EC outside of the junction and fenestra; with AQPs, streamlines emanate from the EC and cause the adjacent streamlines from the junction to slope downwards around the EC-emanating ones as they approach the fenestra. Increasing the AQP1 fraction from 0 to 40\% at fixed \( L_{P_e} \) flattens \( L_{P_t}(\Delta P) \) in the dynamic range 60-100 mmHg since transcellular flow partially relieves the pressure difference across the EC for a given \( \Delta P \). Viewed differently, the shorter flow path traversed by the transcellular vs junctional water flow means less head loss across the EC. Thus one needs a higher \( \Delta P \) to achieve the same trans-EC force/area at the same (e.g., for a single \( k \) for all curves, maximal) SI compression corresponding to the data points. As we shall see, such changes in \( \Delta P \) for maximal compression may be very important. (In the next section we see this effect is much more pronounced with \( L_{P_e} \) not fixed.) Conversely, one can require all curves to look similar by adjusting \( k \) for each data set so they also all have the same \( \Delta P \) for maximal compression (not shown). An AQP1 increase then lowers the trans-EC pressure, i.e., force/area, corresponding to the same \( L_{P_t} \) (i.e., essentially the same compression); this means a lower \( k \). For 40\% AQP1, \( k \) is ((a) 27.98, (b) 19.17, (c) 23.84, (d) 27.9 mmHg), indeed below figure 4’s \( k \) values for 0\% AQP1 (no transcellular flow).
3.3.2 Pressure-dependent intact aortic hydraulic conductivity, $L_{P_t}$, for blocked AQP1s:

Submilimolar concentrations of HgCl$_2$ block trans-AQP1 channels in red blood cells (RBC) (32). Nguyen et al. (19) measured $L_{P_t}$ at three $\Delta P$ values, flushed with 5 $\mu$M HgCl$_2$ to block EC AQP1s, remeasured $L_{P_t}(\Delta P)$, denuded and measured $L_{P_{m+j}}(\Delta P)$, all on the same vessel. Titration curves on RBCs suggests that 5 $\mu$M HgCl$_2$ blocks $<100\%$ and possibly as low as $\sim 1/3$ of AQP1s (37). However, since a transcellular pathway likely consists of more than one AQP1 in series, the number of blocked transcellular pathways may greatly exceeds the number of blocked AQP1s. We get a lower bound on the percent AQP1 contribution to $L_{P_e}$ by assuming 5 $\mu$M blocks all (100%) transcellular pathways and comparing model predictions for various assumed AQP1 fractions with Nguyen et al.’s (19) data. Specifically, for an unblocked $L_{P_e}$ from above with an assumed $L_{P_{EC}}$ (and $L_{P_{nj}}$ from Eq. 20), we shut all AQP1s (set $L_{P_{EC}} = 0$ i.e., 100% blocking), which lowers $L_{P_e}$, and compare the model’s $L_{P_t}(\Delta P)$ prediction with Nguyen et al.’s (19) blocked data. (Note that to predict AQP1 blocking’s effect on $L_{P_t}$, one must use the same $k$ for both unblocked and blocked AQP1 $L_{P_t}$s.) If these $L_{P_t}(\Delta P)$ are above the blocked data ($\leq 100\%$ blocking would worsen the comparison), we raise the AQP1 fraction; if they are below the blocked data, we either decrease the AQP1 fraction or assume $<100\%$ blocking to match the data.

Figure 6 compares Nguyen et al.’s (19) data with the model-generated $L_{P_t}(\Delta P)$ with HgCl$_2$ for various $L_{P_{EC}}$ fractions. The figure includes the intact-unblocked, intact-blocked and denuded vessel data and the corresponding theoretical curves, with the intact-unblocked curve computed for an AQP1 fraction of 40\% of $L_{P_e}$ (Fig. 5). These predictions show a significant decrease in blocked-AQP1 intact aorta $L_{P_t}$ with increased AQP1 fraction, including a small decrease after SI compression. More interesting, the higher this fraction the lower is the critical $\Delta P$ to achieve the force/area for maximal SI compression: the curves in Fig. 6 retain their shape, but shift down (decreased $L_{P_t}$) and to the left as the AQP1 ($L_{P_{EC}}$) fraction that is blocked increases. The curves for 30-40\% AQP1 fraction, 100\% blocked, match the vessel data. Nguyen et al.’s (19) in vitro data show AQP1 blocking reduced water flux across BAECs by $\sim 22.1\pm 6\%$ but AQP1 knockdown lowered RAEC monolayer $L_{P_e}$ by 56.4\%, a small portion of which ($\sim 17\%$) may be due to reduced junctional transport (19); thus 30-40\% AQP1 is not unreasonable.

Experimental predictions: At 60 mmHg, the theory predicts a fully compressed SI with HgCl$_2$, but only a partially compressed SI and no fenestral blockage without it. Direct SI thickness measurement (under way) for vessels fixed under pressure as in (8), either with normal, elevated or blocked AQP1s, will show if, as predicted, there is a large difference at 50 mmHg but not at 100 and 150 mmHg, between aortas fixed with or without HgCl$_2$. Our theory calculates that the force/area acting on the endothelium at 60 mmHg with functioning AQPs is the same - with the same slight SI compression and absence of fenestral blockage - as that acting at 43 mmHg with
blocked AQP1s. But HgCl$_2$ treatment still lowers $L_{P_e}$. The theory thus predicts $L_{P_e}$ at 43 mmHg with HgCl$_2$ is $2.11 \times 10^{-8}$ cm/sec $\cdot$ mmHg, between $L_{P_e}$ with and without HgCl$_2$ at 60 mmHg (1.75 vs $2.55 \times 10^{-8}$ cm/sec $\cdot$ mmHg). Even with HgCl$_2$, lowering $\Delta P$ should sufficiently decompress the SI and raise $L_{P_e}$: the model predicts that, with HgCl$_2$, reducing $\Delta P$ from 60 to 20 mmHg increases $L_{P_e}$ from 1.75 to $2.15 \times 10^{-8}$ cm/sec $\cdot$ mmHg. Nguyen et al. (19) and found $L_{P_e}$ rose to 1.9$\pm$0.2$\times 10^{-8}$ cm/sec $\cdot$ mmHg (SEM) at 20 mmHg with HgCl$_2$, consistent with this prediction.

Nguyen et al. (19) found, with HgCl$_2$, $L_{P_e}$=2.00$\pm$0.28$\times 10^{-8}$ cm/sec $\cdot$ mmHg at $\Delta P$=140 mmHg, slightly (reproducibly, but not statistically significantly) higher than $L_{P_e}$ at 60 and 100 mmHg. Hints of a small rise at high $\Delta P$ also appear in HgCl$_2$-free studies (1, 23, 19). These may simply be the result of added stress on the cannulation ties. Toxicity is unlikely: reducing the Hg$^{++}$-cis bond with 2-mercaptoethanol recovered $L_{P_e}$ values to within 5% of baseline at the tested $\Delta P$=60, 100 mmHg. Any conceivable EC toxicity at 140 mmHg would have caused a far larger $L_{P_e}$ jump than observed. Since HgCl$_2$ raises the force/area on the EC at fixed $\Delta P$, high $\Delta P$ (140 mmHg) and HgCl$_2$ might stretch EC junctions slightly, thus slightly elevating $L_{P_{nj}}$, $L_{P_e}$ and $L_{P_i}$. The model calculates that the force/area on the endothelium with HgCl$_2$ at $\Delta P$=140 mmHg equals that without HgCl$_2$ at $\sim$200 mmHg. $L_{P_i}$ measurement without HgCl$_2$ at 100, 140 and 200 mmHg and with HgCl$_2$ at 140 mmHg on the same vessel would confirm whether without HgCl$_2$ $L_{P_i}$ rises significantly (stretching) at 200 vs140 mmHg and whether $L_{P_i}$ at 140 mmHg with HgCl$_2$ only falls short of $L_{P_i}$ at 200 mmHg without HgCl$_2$ by an amount due to setting $L_{P_{EC}}$ to zero at fixed SI compression. With no change in $L_{P_{nj}}$, the model (AQP1 30-40% of $L_{P_e}$; $L_{P_e}$ derived from Nguyen et al.’s (19) 140 mmHg data) predicts a 200 mmHg no blocker $L_{P_i}$=2.18$\times 10^{-8}$ cm/sec $\cdot$ mmHg.

3.3.3 Effect of AQP1 up-regulation on force/area on EC:

Since decreasing functioning AQP1s lowers the critical $\Delta P$ for SI compression, could increasing functioning AQP1s decompress the SI and substantially raise $L_{P_i}$ in the physiological range? Increased transmural water flow might slow LDL retention kinetics. Figure 7 shows how upregulating AQP1s (transcellular flow) would change the force/area on the ECs. Each curve in figure 7 starts out nearly proportional to $\Delta P$ since $L_{P_i}$ is fixed for each curve and early compression causes no fenestral blocking. At a certain $\Delta P$, SI compression starts to cause fenestral blockage, which lowers $L_{P_{e+i}}$ and $L_{P_i}$ (the intrinsic $L_{P_e}$ is fixed) and nearly balances the higher driving $\Delta P$; this flattens the curve. Beyond maximal SI compression, the geometry and $L_{P_i}$ remain fixed and the material becomes simple, i.e., the transendothelial force/area (proportional to the transendothelial or transmural flow) is linear in $\Delta P$. An increase in functioning AQP1s (transEC flow pathways) shifts the transendothelial force/area curves in Fig. 7 to the right, meaning a given force/area on the EC, e.g., the critical force/area for SI compression, requires a higher, potentially superphysiological, $\Delta P$. This may be beneficial in flushing unbound lipid from the wall.
3.3.4 Effect of AQP1 up-regulation on $L_{Pt}$:

The model predicts (Figure 8) that increasing transcellular transport of an intact vessel wall by increasing the number of functioning EC AQP1s significantly increases $L_{Pt}$ before full SI compression. As Fig. 8 depicts, more AQP1s make the $L_{Pt} (\Delta P)$ curve flatter (as already seen in Fig. 5) and shifts it up and to the right; the inverse of AQP1 lowering seen in Fig. 6. This shift raises the critical $\Delta P$ (for Nguyen et al.’s data (19)) to 90, 95, 104, 110 and 127 mmHg for successive 25% increases in absolute AQP1 expression. Thus the more active the transcellular pathways, the higher the $\Delta P$ needed to achieve the critical force/area for maximal SI compression, i.e., the farther right in $\Delta P$ the shift in $L_{Pt}$’s dynamic range. This prediction suggests a possible intervention: increase vessel wall $L_{Pt}$ by decompressing the SI in the physiological $\Delta P$ range. We anticipate $L_{Pt}$ measurements on vessels with upregulated EC AQP1s will show a significant $L_{Pt}$ rise in the region, $\Delta P \sim 70$-$95$ mmHg, where the dynamic regime shifts and only a small change beyond 110 mmHg, where the SI is fully compressed even with upregulated AQP1.(C. Raval et al., in preparation).

4 CONCLUSION

Water flux across the artery wall plays an important role in prelesion atherosclerosis. Fluid mechanics models for these flows in a layered wall comprised of an endothelium, a compressible subendothelial intima, a fenestrated internal elastic laminar and a media only admit analytic solution for approximate descriptions of the flow in the fenestra (10). By using a numerical procedure that solves for the flow with a more realistic fenestral description, we improved on this approach and confirmed that pressure-induced intima compression can partially block IEL fenestrae and drastically lower wall $L_{Pt}$. Based on recent experiments (19), we then extended this model to include, for the first time, the role of cell membrane AQP1 in transendothelial water transport. We first found that reallocating a portion of the endothelial hydraulic conductivity from the intercellular junction to the cell body smoothed the intima’s pressure-induced compression. Then, rather than reallocating the water permeability, we tested the effects of changing AQP1 number at fixed junction permeability. We thus explained the perplexing HgCl$_2$-induced changes in the pressure-dependence of vessel wall hydraulic conductivity in (19) by showing that an increase (decrease) in the number of transcellular AQP1 pathways shifts to higher (lower) pressures the dynamic pressure range over which $L_{Pt}$ drops from its uncompressed to its maximally compressed subendothelial intima value. Thus blocking AQP1s causes SI compression at lower $\Delta P$ than with normal, functioning AQP1s and, together with the lower intrinsic $L_{Pt}$, significantly reduces $L_{Pt}$ in the shifted pressure regime. The agreement with Nguyen et al. (19) not only supports the hypothesis that AQP1s provide a significant transcellular water transport pathway, but also quantifies this
contribution by estimating that AQP1 is responsible for 30-40% of transendothelial water transport at normal aortic AQP1 levels. We also calculated that the force acting on the endothelium at 60 mmHg with functioning AQP1s is same as that at 43 mmHg for blocked AQP1s. This suggests that the SI in the presence of HgCl$_2$ should decompress at $\Delta P$s below 43 mmHg to a level that the untreated SI is at 60 mmHg (although $L_P$ would still be lower with blocker at 43 mmHg than without blocker at 60 mmHg due to the blocker-reduced $L_P$). Data at 20 mmHg support this prediction. Since it may be clinically more desirable to increase, rather than to decrease $L_P$, we also examined the case of elevated endothelial AQP1 expression. In addition to its prediction of how AQP1 numbers directly affect SI thickness at different $\Delta P$s, a strong test of the model is its prediction that raising endothelial AQP1 drastically increases the critical $\Delta P$ for the $L_P$ drop with SI compression from 80-100 mmHg to well into the physiological regime (>100 mmHg). This could lead to a substantially higher physiological $L_P$ and thus transmural water flow. Higher flows may slow lipid accumulation rates that can lead to early atherosclerotic lesions. Such new understandings of the nature of the transendothelial water flow may suggest new potential therapeutic targets for slowing the early progress of atherosclerosis.

5 ACKNOWLEDGMENTS

We thank the National Institutes of Health (1R01-HL067383) and the National Science Foundation (IOS-0922051, CTS-0077520) for supporting this work.
References


Figure captions:

1. Schematic of periodic local wall unit around a fenestral pore including, from top, endothelial glycocalyx, endothelial cell, subendothelial intima (SI), IEL and media. SI under non-deformable endothelium is compressible; \( L_{i0}^* (L_i^*) \) is the uncompressed (compressed) SI thickness at zero (elevated) transmural pressure. Water enters the SI through junctions/clefts surrounding the EC and the media through the IEL fenestra.

2. Local pressure distributions on the SI side (\( P_i \): the upper curve of a given color) and the medial side (\( P_m \): the lower curve of a given color) of the IEL for different SI thicknesses using Tedgui and Lever’s (30) data with (a) Huang et al.’s (10) series solution using the three approximate boundary conditions and (b) the finite difference solution and exact boundary conditions. \( L_{P_m} \) and \( L_{P_e} \) are given in tables 4 and 5. Fig. (a), 50nm, shows all three approximate fenestral matching conditions: exact pressure matching only at the fenestral center, only on average across the fenestra or at three points in the fenestra. The upper three curves use the most accurate three-point condition. They are similar to the finite difference solution in (b) but shows slight deviations from it even far from the fenestra. Both show a shift of the major pressure drop from the media to the SI as the SI thins.

3. Nonlinear relationship between SI thickness and transmural pressure and comparison with Huang et al.’s (8) experimental measurements of SI thickness in rat aorta fixed \textit{in situ} at different transmural pressures. Tables 4 and 5 give \( L_{P_m} \) and \( L_{P_e} \) for the four data sets. \( L_{i0} \) is initial SI thickness. For each data set, we determine the SI spring constant as explained in the text and plot Eq. 18 with that value to get a continuous curve. All curves show an almost linear initial decline with pressure that quickly levels off as partial fenestral blockage occurs and maximal compression obtains.

4. Comparison of the model’s \( \Delta P \)-dependent (independent) hydraulic conductivity of the intact (denuded) artery wall with the experiments and resulting spring constants, \( k \), from (a) Tedgui and Lever (30), (b) Baldwin and Wilson (1) on rabbit aortas and (c) Shou et al. (23), (d) Nguyen et al. (19) on rat aortas. Diamonds (squares) are experimental data for denuded (intact) ECs; dashed and solid lines are corresponding model predictions. Tables 4 and 5 give \( L_{P_m} \) and \( L_{P_e} \) for each of these data sets. The data, and thus the derived \( k \), in (c) are far different from the other experiments. The high \( k \) in (a) means a high \( \Delta P \) is needed for maximum compression; the paucity of data means a lower \( k \) and \( \Delta P \) would also fit.

5. Effect of various AQP1 fractions (open and functioning; \( L_{PEC} \)) at fixed total \( L_{Pe} = 8.29 \times 10^{-8} \) cm/sec \cdot mmHg and \( k \) on the \( \Delta P \)-dependent hydraulic conductivity of an intact artery wall and comparison with Nguyen et al.’s (19) experimental data. For each \( L_{Pe} \) curve, Eq. 20
determines $L_{P_{nj}}$. Symbols: experimental data, Lines: model predictions. $L_{P_{m}}$ and $L_{P_{e}}$ determine the low $\Delta P$ and the experiment the high $\Delta P$ plateaus, both the same for all curves. The more of $L_{P_{e}}$ that $L_{P_{EC}}$ accounts for, the milder the drop between plateaus and thus the higher the $\Delta P$ for maximal SI compression.

6. Effect of AQP blocking for different AQP1 fractions of $L_{P_{e}}$ at fixed, unblocked $L_{P_{e}}$ on the $\Delta P$-dependent hydraulic conductivity of an intact artery wall, including a comparison with Nguyen et al.’s (19) experimental data. Symbols: experimental data, Lines: model predictions. Blocking AQP1s (solid lines) for a given AQP1 fraction leaves $L_{P_{nj}}$ unchanged, but shuts off (sets to zero) $L_{P_{EC}}$ and thus lowers $L_{P_{e}}$. $L_{P_{nj}}$ for all curves is the same as that in Fig. 5; dashed blue curve is the same as the 40% AQP curve in Fig. 4d. The larger the AQP fraction of $L_{P_{e}}$, the larger the HgCl$_2$ effect; 30-40% AQP1 fit the data best.

7. Effect of increasing functioning AQP1 number, i.e., fraction of $L_{P_{e}}$, on the force/area acting on the endothelium as a function of $\Delta P$. Baseline results assume that AQP1s are 40% of $L_{P_{e}}$. The corresponding $L_{P_{EC}}=3.32 \times 10^{-8}$ cm/sec · mmHg (Eq. 20), $L_{P_{nj}}=2.99 \times 10^{-5}$ cm/sec · mmHg and $k =27.9$ mmHg; $L_{P_{nj}}$ and $k$ are fixed for different $L_{P_{EC}}$ fractions of $L_{P_{e}}$. All curves rise linearly as rising $\Delta P$ compresses the linear spring until partial fenestral blockage redistributes that total $\Delta P$. Break in slope of all curves occurs at the same force/area on the endothelium, indicating maximal compression, after which no further compression occurs, the material becomes simple and the curves again rise linearly.

8. Effect of increasing the number of functioning AQP1s, i.e., $L_{P_{EC}}$, at fixed $L_{P_{nj}}$, on $L_{P_{t}}$ of an intact artery wall. Baseline results assume that AQP1s are 40% of $L_{P_{e}}$; from Eq. 20, $L_{P_{EC}}=3.32 \times 10^{-8}$ cm/sec · mmHg and $L_{P_{nj}}=2.99 \times 10^{-5}$ cm/sec · mmHg. $L_{P_{nj}}$ and $k$ (=27.9 mmHg) are the same for all curves. Increasing AQP1 shifts the critical $\Delta P$ for SI compression and thus $L_{P_{t}}$’s dynamic range to higher $\Delta Ps$ - potentially into the physiological regime. A decompressed SI substantially raises $L_{P_{t}}$. 

26
Figure 1
Figure 2

(a) $P(r_f, \Pi, \Pi_m)$ (at $z=0$)
(b) $P(r_f, \Pi, \Pi_m)$ (at $z=0$)
Figure 3

Transmural Pressure (mmHg)

$L_i/L_{i0}^*$ (for $L_{i0}^*$ = 500 nm)

- Huang et al. (12)
- Tedgui & Lever data (32)
- Baldwin & Wilson data (1)
- Shou et al. data (25)
- Nguyen et al. data (20)

Transmural Pressure (mmHg)
Figure 4

(a) $L_p \times 10^8$ (cm/s/mmHg) vs. Transmural Pressure (mmHg)

- $k = 32.7$ mmHg

(b) $L_p \times 10^8$ (cm/s/mmHg) vs. Transmural Pressure (mmHg)

- $k = 23.68$ mmHg

(c) $L_p \times 10^8$ (cm/s/mmHg) vs. Transmural Pressure (mmHg)

- $k = 27.66$ mmHg

(d) $L_p \times 10^8$ (cm/s/mmHg) vs. Transmural Pressure (mmHg)

- $k = 30.6$ mmHg
Figure 5

![Graph showing transmural pressure vs. Lp](image)

- **Denuded EC**
- **Intact EC (AQPs open)**
- **0% AQPs**
- **20% AQPs**
- **30% AQPs**
- **40% AQPs**
Transmural Pressure (mmHg)

Denuded EC
Intact EC (AQPs open)
AQPs blocked

40% AQPs(all open), \( k=27.9 \)
20% AQPs(all blocked), \( k=29.2 \)
30% AQPs(all blocked), \( k=28.5 \)
40% AQPs(all blocked), \( k=27.9 \)

Figure 6
Figure 7
Figure 8
Glossary:

\( a^* \)  
Effective radius of proteoglycan aggregates

\( h_j \)  
Ratio of thickness of region \( j \) to radius of fenestral pore

\( j \)  
Region of artery wall (\( j = g, i, m \) for GX, SI and Media respectively)

\( J_v \)  
Water flux

\( k \)  
Elastic coefficient of SI

\( K_{P_j} \)  
Darcy permeability of region \( j \)

\( L_{i0}^* \)  
Thickness of SI at zero luminal pressure

\( L_{ic}^* \)  
Critical thickness of SI layer

\( L_j^* \)  
Thickness of region \( j \)

\( L_p \)  
Hydraulic conductivity

\( L_{pe} \)  
Intrinsic hydraulic conductivity of the endothelium

\( L_{pEC} \)  
Hydraulic conductivity of the endothelium attributed to its AQP1s

\( L_{pe+i} \)  
Hydraulic conductivity of the endothelium + SI

\( L_{pi} \)  
Hydraulic conductivity of SI

\( L_{pI} \)  
Hydraulic conductivity of IEL

\( L_{pm} \)  
Hydraulic conductivity of media

\( L_{pm+i} \)  
Hydraulic conductivity of IEL + media

\( L_{pj} \)  
Hydraulic conductivity of normal junction surrounding an EC

\( L_{pt} \)  
Total hydraulic conductivity of the vessel wall

\( P_j^* \)  
Dimensional pressure in region \( j \)

\( P_j \)  
Dimensionless pressure in region \( j \)

\( \bar{P}_j^* \)  
Dimensional average pressure in region \( j \)

\( \bar{P}_j \)  
Dimensionless average pressure in region \( j \)

\( P_L^* \)  
Lumen pressure

\( P_{Lc}^* \)  
Dimensional critical pressure

\( r \)  
Dimensionless radial coordinate

\( r^* \)  
Dimensional radial coordinate

\( r_f^* \)  
Radius of fenestra
\( U_j^* \) Dimensional lateral velocity in region \( j \)
\( U_j \) Dimensionless lateral velocity in region \( j \)
\( R^* \) Dimensional radius of EC
\( R \) Dimensionless radius of EC
\( V \) Velocity vector
\( W_f^* \) Dimensional water velocity through fenestra
\( W_j \) Dimensionless normal velocity in region \( j \)
\( z \) Dimensionless normal coordinate
\( z^* \) Dimensional normal coordinate
\( \Delta R^* \) Dimensional width of normal junction
\( \Delta R \) Dimensionless width of normal junction
\( \Delta P \) Dimensionless pressure drop across a membrane
\( \Delta \pi \) Dimensionless osmotic pressure difference across a membrane
\( \mu \) Viscosity of fluid
\( \xi_i^* \) Dimensional radius of periodic wall unit
\( \xi_i \) Dimensionless radius of periodic wall unit
Table 1: Measured hydraulic conductivity with and without endothelium in rabbit and rat aortas

<table>
<thead>
<tr>
<th>Data</th>
<th>Species</th>
<th>Pressure (mmHg)</th>
<th>Hydraulic Conductivity $\times 10^8$ (cm/s·mmHg)</th>
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<tbody>
<tr>
<td>Tedgui &amp; Lever (30)</td>
<td>Rabbit</td>
<td>70</td>
<td>4.00±1.31</td>
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<td>5.27±0.84</td>
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<td>Baldwin &amp; Wilson (1)</td>
<td>Rabbit</td>
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<td>150</td>
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<td>10.53±2.83</td>
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<td>Shou et al. (23)</td>
<td>Rat (Sprague-Dawley)</td>
<td>60</td>
<td>4.69±1.2</td>
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<td></td>
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<td>Nguyen et al. (19)</td>
<td>Rat (Sprague-Dawley)</td>
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<td>2.10±0.23</td>
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<td>3.86±0.37</td>
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Values are mean ± SD
Table 2: Nguyen et al.’s data (19): Effect of AQPI blocking on hydraulic conductivity of intact artery wall

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<thead>
<tr>
<th>Pressure (mmHg)</th>
<th>Hydraulic Conductivity, $L_{P1} \times 10^8$ (cm/s·mmHg)</th>
<th>Unblocked AQPs</th>
<th>Blocked AQPs</th>
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<tr>
<td>60</td>
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<td>140</td>
<td>2.10(\pm)0.23</td>
<td>2.00(\pm)0.28</td>
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Values are mean ± SD
Table 3: Parameters and constants used in the model

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<th>Constant/Parameter</th>
<th>Value</th>
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<td>(a^*, \text{nm})</td>
<td>2.37</td>
<td>a</td>
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<tr>
<td>(L_g^*, \text{nm})</td>
<td>200</td>
<td>(40)</td>
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<tr>
<td>(L_{i0}^*, \text{nm})</td>
<td>500</td>
<td>(10)</td>
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<tr>
<td>(L_m^*, \text{μm})</td>
<td>141(rat), 125(rabbit)</td>
<td>(10, 23)</td>
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<td>(R^*, \text{μm})</td>
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<td>(35)</td>
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<td>(r_i^*, \text{μm})</td>
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<td>(10)</td>
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<td>(\Delta R^*, \text{nm})</td>
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<td>(35)</td>
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<tr>
<td>(K_{P_g}, \text{cm}^2)</td>
<td>(4.08 \times 10^{-14})</td>
<td>b</td>
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<tr>
<td>(K_{P_i}, \text{cm}^2)</td>
<td>(2.20 \times 10^{-12})</td>
<td>(10),c</td>
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<td>(\delta_{CG}, \text{nm})</td>
<td>170.35</td>
<td>d</td>
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<td>(\delta_{PG}, \text{nm})</td>
<td>40</td>
<td>(10)</td>
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<tr>
<td>(\epsilon_{CG0})</td>
<td>0.95</td>
<td>(9)</td>
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<tr>
<td>(\epsilon_{PG0})</td>
<td>0.9883</td>
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<tr>
<td>(\mu, \text{kg/m \cdot sec})</td>
<td>(7.2 \times 10^{-4})</td>
<td>(12)</td>
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a: calculated using fiber matrix theory as explained in (9)

b: estimated as explained in the text

c: uncompressed SI value at zero transmural pressure; corresponding values for compressed SI \((L_i^*)\) are calculated as explained in the text and in (10)

d: calculated by assuming hexagonally packed collagen fibers of radius 20 nm (9) with an initial volume fraction of 5% (9)
Table 4: Converged $L_{P_m}$ values

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<tr>
<th>Data</th>
<th>$L_{P_m} \times 10^8$ (cm/s·mmHg)</th>
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<tbody>
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<td>Tedgui &amp; Lever (30)</td>
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<td>10.37</td>
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<td>Nguyen et al. (19)</td>
<td>7.50</td>
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Table 5: Converged $L_{P_e}$ values

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<th>Data</th>
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<tr>
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<td>35.80</td>
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<td>16.52</td>
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<td>Nguyen et al. (19)</td>
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