EFFECTS OF PULSATION FREQUENCY AND ENDOTHELIAL INTEGRITY ON ENHANCED ARTERIAL TRANSMURAL FILTRATION PRODUCED BY PULSATILE PRESSURE

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The role of the endothelium in regulating transmural fluid filtration into the artery wall under pulsatile pressure, and the effects of changes in pulsatile frequency on filtration, have received little attention. Previous experiments (1) demonstrated significantly increased filtration after initial onset of pulsatile pressure compared to that predicted using parameters measured under steady pressure. In order to determine the role of the endothelium in this phenomenon, the following experiments were performed on five New Zealand White rabbits (anesthetized with 30 mg/kg pentobarbital sodium). One of each pair of carotid arteries was de-endothelialized and filtration measurements under steady and pulsatile pressure compared with those made in intact vessels (1). To determine the effect of increasing pulsatile frequency on arterial filtration, transmural filtration was measured using pulsatile pressure frequencies of 1 Hz, followed by 2 Hz, in another set of intact arteries (6 arteries, 3 animals). For de-endothelialized vessels, the initial increase in filtration after onset of pulsatility was similar to that observed in intact vessels, but the subsequent reduction in filtration was less abrupt. When pulsatile frequency was increased from 1 to 2 Hz in intact arteries, an initial increase in filtration was observed, similar to that obtained after onset of pulsatile pressure subsequent to a steady pressure. The observed responses of arteries to pulsatile pressure, with and without endothelium, or undergoing a frequency change, suggest a dynamic role for the endothelium in regulating transvascular transport in vivo.

Keywords: Interstitial hydration, De-endothelialized vessels, Convective transport
INTRODUCTION

Convective fluid flow through the artery wall has been demonstrated to greatly facilitate the transport of macromolecules into the arterial intima (8). Accumulation of macromolecules, such as low density lipoprotein, in the arterial intima can lead to formation of fatty streaks, a precursor to atherosclerosis. Previous investigators have examined the variation of hydraulic conductance, $L_p$, a parameter of convective fluid transfer, at different steady hydrostatic pressures (4). Our subsequent experiments showed that the onset of a pulsatile pressure (1 Hz), superimposed on a baseline pressure of 60 or 80 mmHg, produced a large, but transient, burst of increased filtration (1). Over time, the transient burst of filtration was dampened and filtration continued at a lesser rate, this rate still being three times greater than that predicted by steady state measurements (1).

Studies have shown that the endothelium plays a significant role in controlling fluid filtration through the artery wall under steady-state pressure. These experiments (18, 19) demonstrated that the endothelium accounts for about half of the hydraulic resistance of the whole vessel wall. Therefore, one goal of the present study was to evaluate the role of the endothelium in the initial increase, and subsequent decrease, in filtration following the onset of pulsatility. It is important to examine arteries when they are exposed to pulsatile pressure with variations in frequency because in vivo, pulsatile pumping of the heart causes cyclic changes in arterial transmural pressure, creating rapid shifts in arterial wall stress with possible changes in wall transport properties. In addition, the pulsatile frequency, although fairly constant when the body is at rest, increases abruptly with
sympathetic stimulation. Therefore a second goal of the present study was to determine whether the transient increase in filtration observed in the previous study (1) still occurred following an increase in frequency of an already established pulsatile pressure regime.

METHODS AND MATERIALS

Experiments to compare changes in fluid influx into the artery wall, following onset of pulsatile pressure in intact versus de-endothelialized vessels, were performed on five male New Zealand White rabbits (Av. weight 3.8 kg.) anesthetized with sodium pentobarbital (30 mg/kg iv administered). Three other male New Zealand White rabbits (average weight 3.3 kg.) were similarly anesthetized and used for the experiments to examine the effects of frequency changes in pulsatile pressure. All experiments were conducted within animal welfare regulations and guidelines for the U.S.A. under IACUC approval. The following procedures were performed.

Protocol for Measuring Fluid Flux in De-endothelialized Vessels

To measure fluid filtration in the de-endothelialized arteries under pulsatile pressure, carotid arteries were cannulated and excised without depressurization or change in length. These carotid arteries were the pairs of those used in the previous study (1), and so the intact arteries from the previous study served as a control group in the current experiment. After excision, the arteries used in the present study were placed in the
experimental apparatus (figure 1) and each was de-endothelialized by passing an air bubble along its length.

Hydraulic conductance ($L_p$) at a steady pressure of 60 mmHg was measured in each de-endothelialized artery. Then a pulsatile pressure (10-14 mmHg) was superimposed upon a steady pressure of either 60 or 80 mmHg, and, after the passage of a given set of pressure pulses, the resultant fluid filtration during that time period was measured. A pressure transducer (Viggo-Spectramed) was used to record the pressure changes over time. Simultaneous measurement was made of the arterial distension, before, during and after application of the pulsatile pressure, using Optical Coherence Tomography (OCT), so that the filtration measurements could be corrected for any residual distension due to viscoelastic effects. Comparisons were made of the observed experimental fluid filtration into the de-endothelialized artery wall (corrected for residual distension) to the predicted filtration (calculated from the measured steady pressure $L_p$ and the time-averaged pressure estimated using the recorded sequence of pressure pulses) and to the previously measured values for intact vessels (1).

**Protocol for Measuring Fluid Flux in Vessels Receiving Change in Pulsatile Frequency**

In a second set of anesthetized animals, each pair of carotid arteries was surgically prepared (cannulated and then excised) such that the endothelium remained intact. The hydraulic conductance ($L_p$) at a steady pressure of 60 mmHg was measured in each vessel. Next, an initial pulsatile pressure regime (10-14 mmHg pulsatile pressure) was
superimposed on the baseline pressure (60 mmHg)), after which the frequency of the pressure pulses was doubled (from 1 Hz to 2 Hz) for a given set of pulses and the resultant fluid filtration was measured. A pressure transducer was used to measure and record the pressure changes over time, and OCT was used to measure arterial distension before, during, and after the frequency variation.

The observed experimental fluid filtration into the artery wall (corrected for residual distension) at the 2 Hz pulsatile frequency subsequent to a ‘baseline’ of 1Hz frequency, was compared to that predicted for steady-state pressure, and to the values previously measured in arteries which experienced steady pressure followed by the onset of 1 Hz frequency pressure pulses (1). The predicted steady state values for filtration were calculated from the measured steady pressure $L_p$ and the time-averaged pressure of the pulse train.

**Surgery**

During a previous study (1), both rabbit carotid arteries were surgically removed from each animal and one vessel was used to determine the effects of pulsatile pressure on fluid filtration. The second carotid artery from each pair was used in the present study to determine the role of the endothelium in response to pulsatile pressure. Both vessels were cannulated, pressurized to 60 mmHg with phosphate buffered saline (PBS, 4.0% bovine serum albumin, pH 7.4), excised, and then stored briefly in PBS before their use (1). Before excision, any branches from the main artery had been ligated. A stainless steel holder clipped to each cannula maintained the excised arteries at their original
physiological lengths. The cannulated segment was placed into the apparatus shown in Figure 1 and the segment was perfused with PBS containing 0.03% Trypan blue. Inclusion of the Trypan blue in the perfusate allowed for detection of any unligated branches when the vessels were pressurized and the outlet closed. The arterial segment was then de-endothelialized by passing a small air bubble through its length. Previous studies using electron microscopy have shown that this method removes the endothelium without damaging the underlying internal elastic lamella (3). Next, the segment was preconditioned by repeatedly pressurizing and depressurizing between 10 and 100 mmHg, following a previously established procedure (4).

**Steady-State L_p Measurement**

An air-bubble introduced into a length of transparent plastic tubing attached to the arterial inlet cannula was used to measure the artery L_p under steady pressure, following a previously established procedure (4). Using this technique, a step-change in pressure was imposed within the arterial lumen, with the arterial outflow occluded. The air bubble moved due to a combination of pressure-induced arterial distension and transmural filtration. After a few minutes the bubble moved with a constant velocity corresponding to the transmural fluid filtration rate. This value was then is used to obtain L_p (see equation 1 below (4, 14)):

\[
\text{Rate of filtration} = L_p A_s \overline{P} = \pi r^2 v \quad \text{(Equation 1)}
\]
where $A_s =$ artery surface area, $\bar{P} =$ time-averaged transmural pressure, $r =$ radius of plastic tube and $v =$ bubble velocity

This calculation assumes that there is no osmotic pressure difference across the vessel wall. Earlier calculations (17) demonstrated that the colloid osmotic pressure difference in intact vessels was less than 10 mmHg for a vessel immersed in a 2% albumin solution with a 4% albumin solution perfusing the vessel. With a 4% albumin solution used for both immersion and as a perfusate, as used in the present study, the colloid osmotic pressure difference would be even smaller, probably an order of magnitude less than the hydrostatic pressure difference. It might be argued that pressurization of the artery for the 10 to 20 minute time period required for measurement of $L_p$ might hydrate the arterial wall. However, when Tedgui and Lever (17) tested for changes in extracellular space (ECS) and water content (WC) in intact and de-endothelialized vessels pressurized at 70 mmHg for 90 minutes, no significant increases were observed. Therefore, it would not be expected that the steady-state calculation would be affected by fluid accumulation.

**Creation of Pressure Regimes**

**Pressure Pulse Creation**

Pulsatile pressure was created in the arterial segments with a Harvard Apparatus pump (model 1421 Pulsatile Blood Pump). The same apparatus described in the previous study (1) was used to create a high resistance outlet with oscillatory pressure, but without net fluid flow, in series with the cannulated end of the artery (see Figure 1). A Viggo-Spectramed pressure transducer was attached to the cannula outlet at the other end of the
artery. As in previous experiments, baseline pressure was established in a pressure reservoir connected to the arterial inlet, using a sphygmomanometer bulb with attached mercury manometer, and the pressure transducer was calibrated at the start of each experiment. The Harvard pump was activated and six sets of 5-pulse trains, followed by six sets of 20-pulse trains at 60 and 80 mmHg baseline pressures at a frequency of one pulse per second were applied to the carotid arteries. In between the pulse-trains there were 10-20 second periods of steady pressure in order to allow the apparatus to be reset. Data on the fluid flux into the artery during these interim periods were not obtained.

Synchronized data outputs were recorded from the transducer and imaging system throughout each pulse-train. As was seen previously (1), the bubble oscillated back and forth with each pressure pulse, and was often shifted toward the vessel after passage of the pulse train. The motion of the bubble during each pulse-train was videotaped to determine the initial and final positions of the bubble, and the total time of oscillatory motion. The net movement of the bubble towards the artery arose from the sum of any residual arterial distension and the fluid filtered from the artery lumen. From these measurements, and knowing the geometry of the tube containing the bubble, the total volume of fluid filtering through the artery wall for a given pulse train, could be calculated. This value had to be corrected for any residual distension that may have occurred, as described below. The corresponding fluid loss predicted to occur had the pressure been steady, rather than pulsatile, was found using equation (2):

\[
P\text{redicted fluid loss} = L_p A_\phi \bar{P} t \quad (\text{Equation 2})
\]
Where \( L_p \) = previously measured hydraulic conductance at steady pressure, \( A_s \) = artery surface area, \( \bar{P} \) = time-averaged transmural pressure and \( t \) = duration of oscillation. Thus, the filtration measured under pulsatile pressure could be compared with that predicted for the same time-averaged steady pressure.

**Measurement of Residual Distension**

To determine the fluid loss from the arterial lumen it was necessary to measure the residual distention using Optical Coherence Tomography (OCT) as performed in a prior study (1). Details concerning the OCT system have been described previously (5, 13). The current system consists of a short-coherence length light-source (super-luminescent diode, 1290 nm center wavelength and 49 nm bandwidth) allowing for a 16-\( \mu m \) coherence length (and thus axial resolution) in air. Assuming a tissue index of refraction of 1.4 (6), then tissue axial resolution is approximately 11 \( \mu m \). The sample arm light is focused to a 15-\( \mu m \) spot on the tissue and an image through the artery wall is obtained by collecting and analyzing the scattered light from the artery. Diameter measurements made using OCT have, at most, one-ninth of the error of previous diameter measurements made using a vernier caliper accurate to 0.1 mm (4). Furthermore, the OCT system measures the actual inner artery diameter, rather than the external diameter (3, 4, 17). In these experiments the system was used to create two-dimensional OCT images (m-scans; i.e., functions of depth and time with time resolution of 100 msec (10 A-scans/sec.)) so as to determine variations in internal diameter caused by pulsatile pressure.
Volume displaced by residual distension ($V_{res}$) is given by:

$$V_{res} = \left(\frac{\pi l}{n^2}\right) (D_f^2 - D_0^2) \quad \text{(Equation 3)}$$

where $l$ is the artery length and $n$ is the index of refraction. Diameter measurements from OCT ($D_o$ and $D_f$) are Optical Path Length measurements and must be divided by the index of refraction to obtain the “true” diameters.

**Changing Frequency of the Pulsatile Pressure**

To establish an initial pulsatile frequency in the artery, a baseline pressure of 60 mmHg was imposed, and then the pump was used to create a pulse-train consisting of 150 continuous pressure pulses at an amplitude of 10-14 mmHg (added to the baseline pressure) and a frequency of 1 Hz. At 60-mmHg baseline pressure, after 150 total pulses, the filtered volume rate settled to a “steady” value (estimated $3.9 \times 10^{-5}$ cm$^3$/sec.) after the initial increase, and no further spurts occurred (1). Next, a set of higher frequency (2 Hz) 5-pulse trains was generated in the artery. The whole procedure was repeated but with 20-pulse trains. Therefore as an example, the artery began at a steady-state 60 mmHg pressure, received an initial 150 pulse pressure train ($(5 \text{ pulses x 6}) + (20 \text{ pulses x 6})$) at set amplitude and frequency (10-14 mmHg, 1 Hz), and then was allowed to stabilize at steady pressure temporarily to measure the initial bubble position. Next the artery received a 5-pulse pressure train at 2 Hz frequency, the experimental filtration volume was measured, and then the process was repeated for five more 5- pulse-trains. After
another 150 pulse-train set, at 1 Hz, the artery was exposed to a set of six 20-pulse double frequency pulse-trains.

During the higher frequency pulse-trains, the pump plunger was forward in its cylinder when the baseline 60 mmHg pressure was set. This was necessary in order to discern that the plunger had started and stopped in the same position, especially when using a higher pulse frequency. The effect of having the plunger forward is that when the pump is engaged and the plunger retracts, the pulsatile component is subtracted from the baseline pressure. Therefore, the pulse train appears inverted. Physiologically, however, this is akin to measuring the diastolic pressure in an artery previous to the systolic pressure.

Statistics

Error bars represent standard errors. The experimental volume corrected for residual distension, averaged over the six 5-pulse values between 5 and 30 pulses, was compared to the corresponding steady-state value using the Student t-test (p<0.05), after assuring a normal distribution of the values using the Kolmorogov-Smirnov test. The 5 – 30 pulse mean experimental value was also compared to the average of the experimental values obtained between 50 and 150 pulses (six 20-pulse trains), the latter parameter being divided by four to account for the fact that these were 20-pulse trials rather than 5-pulse trials. The various plots were tested for normality, checked for equal variances, and then linear regression was performed where possible. When linear regression lines could be found between two regions, F-tests were then performed to test whether the regions
yielded significantly different linear regression lines. Any values that did not satisfy normality were tested using the Wilcoxon Rank-Sum test.

RESULTS

Hydraulic Conductance

For the de-endothelialized arteries, the hydraulic conductance ($L_p$) of each artery was measured experimentally under steady pressure so as to estimate the predicted volume of fluid filtered through the artery wall. An average value of $6.03 \pm 3.88$ (SD) $\times 10^{-7}$ cm sec$^{-1}$mmHg$^{-1}$ ($n=5$) was obtained and this was significantly greater ($p < 0.05$) than the average $L_p$ of intact arteries, $1.49 \pm 1.13$ (SD) $\times 10^{-7}$ cm sec$^{-1}$mmHg$^{-1}$ ($n=6$) (1). For the arteries that were later exposed to a frequency change, the average steady-state $L_p$ was $0.956 \pm 0.482$ (SD) $\times 10^{-7}$ cm sec$^{-1}$mmHg$^{-1}$ ($n=5$), which was not significantly different from the previous values (1). The mean $L_p$ value of the de-endothelialized vessels was almost 3 to 4 times that of the intact, which is consistent with values in the literature. Tedgui and Lever (16) quote values of $L_p$ in damaged rabbit aortas twice that of normal rabbit aortas. The predicted filtered volume for the de-endothelialized vessels was found at each time point using the steady-state $L_p$ values. These values were, on average, approximately three times those of the steady-state predicted volumes of the intact arteries (not shown).
Filtration in De-endothelialized Vessels

Figures 2 and 3 show the cumulative experimental volumes for the de-endothelialized pulsatile vessels (experimental volume is defined hereafter as the volume entering the artery, as measured from the bubble shift forward after a pulse train minus the volume accounted for by the residual distension), compared to: (i) the predicted cumulative steady-pressure filtration volume for de-endothelialized vessels (hereafter referred to as de-endothelialized steady-state vessels), and (ii) the cumulative experimental volumes for the intact pulsatile vessels (intact vessels, data from (1)). As in the prior study, “Cumulative Volume” referenced in Figures 2, 3, and 4 is the summed volume, either experimental or predicted (steady-state) volumes, over time (i.e., Experimental Cumulative Volume, 20 seconds, is the sum of the Experimental Volume values at 5, 10, 15, and 20 seconds). Time-scales in Figures 2 through 4 represent the integrated duration of the pulse trains themselves (Ten pulses are the sum of two five-pulse trains, so the artery experiences approximately a total of 10 seconds of pulsatile pressure).

Graphs are labeled either 60 or 80 mmHg (in Figures 2 and 3, respectively) depending on the baseline pressure used experimentally. As shown in figures 2, 3, and 4, the parameters compared were: (i) the cumulative experimental fluid volumes resulting from 5–30 pulses in de-endothelialized vessels versus those from 50-150 pulses in the same vessels, (ii) the cumulative experimental fluid volumes as described for (i) but versus the estimated steady pressure values in each case, and (iii) as for (ii) but versus the values obtained from intact vessels over the two corresponding ranges of pressure pulses.
Linear regressions, where possible, have been summarized in Table 1 for the 5-30 and the 50-150 pulse-regions of figure 2. Since these are cumulative volumes, it is difficult to predict even the steady-state slope value without linear regression. The steady-state plots were calculated for each trial and then averaged and accumulated on a point-by-point basis, using the time-averaged pressure measurements made during each experimental trial, the $L_p$ value found prior to instigation of the pulsatile pressure, and the actual time of oscillation for each trial run. Linear regression of the de-endothelialized steady pressure and the de-endothelialized pulsatile plots showed that from 5-30 pulses, the slope of the de-endothelialized pulsatile values exceeded the slope of the de-endothelialized steady-state values by a factor of 5.3 ($15.6 \times 10^{-5}$ cm$^3$/sec. versus $2.9 \times 10^{-5}$ cm$^3$/sec., respectively).

Linear regression of the same plots from 50-150 pulses showed that the slope of the de-endothelialized pulsatile values was approximately four-fold that predicted for de-endothelialized steady-state ($11.2 \times 10^{-5}$ cm$^3$/sec. versus $2.7 \times 10^{-5}$ cm$^3$/sec., respectively). F-tests demonstrated significant differences between the slopes of the de-endothelialized pulsatile values and the de-endothelialized steady-state values, for both 5-30 pulses and 50-150 pulses. However, the same test did not show a significant difference between the slopes of the de-endothelialized pulsatile values and the intact pulsatile values at 5-30 pulses even though the de-endothelialized pulsatile and the intact pulsatile values were significantly different at 50-150 pulses. A point-by-point comparison of the intact and de-endothelialized pulsatile results showed a significant difference between the two plots.
at each point from 50–150 pulses (by the Wilcoxon Rank Sum test (15)). Hence, the original assumption (1) that the endothelium plays only a minor role in the production of the transient burst of filtration after onset of pulsatile pressure appears to be accurate but only for the initial 30–50 pulses. As the number of pulses approaches 150, the endothelium appears to play some role in returning fluid filtration closer to initial values.

Figure 3 shows cumulative volumes versus time scatter-plots for the same vessels as in Figure 2, except that these vessels were tested at 80-mmHg baseline pressure. Table 2 summarizes the linear regression slope values at 5-30 pulses and 50-150 pulses. The scatter-plots for the intact pulsatile and the de-endothelialized pulsatile volumes followed a similar trend as for the 60-mmHg plot, but F-tests between the de-endothelialized pulsatile and the intact pulsatile vessels from 50 to 150 pulses did not show a significant difference. However, the point by point comparison over 50–150 pulses, using the Wilcoxon Rank Sum test, indicated significant differences between the intact and de-endothelialized pulsatile vessels at 50, 90, 110, and 130 total pulses. There was no significant difference between the slopes of the de-endothelialized pulsatile vessels and the intact pulsatile vessels at 5-30 pulses. These results support the finding that the endothelium does not appear to regulate the transiently increased fluid filtration into the artery wall until about one minute after the onset of pulsatile pressure.

**Filtration in Vessels Undergoing a Change in Pulsatile Frequency**

Changing the pressure pulse frequency from one to two Hz produced the results shown in figure 4. This increase in pulsatile frequency gave a transient burst of filtration similar to
that found when a one Hz pulsatile pressure was superimposed on a steady pressure (1). Figure 4 also shows the cumulative results from intact pulsatile arteries that had been subjected to only a 0-1 Hz pulse frequency change, and the steady-state cumulative volumes calculated over time periods equivalent to those for the 1-2 Hz frequency change pulse trains (labeled equivalent steady-state volumes). The graph of the 1-2 Hz frequency change vessels displayed the following characteristics: there was an initial region with an apparent transient burst of filtration from 2.5 to approximately 15 seconds, followed by a region of lower filtration from 25-75 seconds. Due to the non-linearity of the plots in figure 4, F-tests were not performed. The values of cumulative volume at 5-30 pulses for intact pulsatile vessels (0–1 Hz change) were significantly smaller than those for the (1-2 Hz change) frequency vessels (Student t-test). The values at 50-150 for the intact pulsatile vessels (0-1 Hz change) were also significantly smaller than the corresponding values for the (1-2 Hz change) vessels.

**Calculation to Determine Whether Increased Fluid Influx Remains in Interstitium**

One question posed by this study is whether the excess fluid entering the arterial intima, subsequent to onset of pulsatile pressure, filters all the way through the artery wall or just remains within the interstitium. To answer this question, a calculation was performed to determine the increase in medial wall thickness that would result if the accumulated volumes of the intact pulsatile vessels shown in figures 2 and 3 remained in the interstitium. At 60 mmHg, the accumulated volume eventually reaches a maximum value of 0.0105 cm³. The formula for the change in volume, $V_{fill}$, corresponding to a change in wall thickness is given in equation (4):
\[ \Delta V_{\text{fill}} = 2\pi R_o l \Delta t \] (Equation 4)

Using the values of \( R_o \) (average artery radius) = 1.0 mm, \( l \) (average artery length) = 1.8 cm., and \( t \) (average artery thickness) = 0.14 mm with a change in \( V_{\text{fill}} \) of 0.0105 cm\(^3\), gives a thickness increase of approximately 92 microns, or a 66% increase in thickness, which should be visible under OCT (1). At 80 mmHg the accumulated volume increases to 0.208 cm\(^3\), which would correspond to a thickness change of 180 microns, or a 131% thickness increase. Such increases in vessel thickness were not observed, indicating that some fluid must have filtered all the way through the artery walls. In addition, these estimated volumes of fluid influx equal or exceed the measured medial water content of the rabbit carotid artery (2), so fluid must, at least at 80 mmHg, be exiting from the adventitial side of the artery.
DISCUSSION

Our previous work (1) raises the question of the role that the endothelium plays in the two phases of filtration into the artery wall, following onset of pulsatile pressure. The results of the present study suggest that, with pulsatile pressure, the endothelium plays a significant role in controlling filtration through the artery wall at later stages after the initial onset of pressure. At first the de-endothelialized arteries have similar filtration characteristics to intact arteries, suggesting that the endothelium may not play a role immediately after a change in the time-varying characteristics of the pressure regime. A comparison of the slopes of the graphs in figure 3 shows that the entire de-endothelialized plot is well modeled by a line passing through the origin, whereas the later points on the intact vessel plot follow a line with a y-intercept greater than zero. The initial slope of the plot for intact arteries is noticeably steeper than the slope at later time points. This change in slope suggests that at some point about 50 to 100 seconds after the onset of pulsatile pressure, the endothelium alters the filtration characteristics of the vessels. The question then arises: what mechanism exists that can allow the endothelium to change its permeability on a time-scale that is of the order of two minutes? One way the endothelial cells could directly alter the filtration characteristics of the artery wall would be to alter the intimal resistance to water flux. Another possible way for endothelial cells to indirectly affect the filtration would be by altering the resistance of the arterial media through release of nitric oxide or autocoids, such as endothelin-1.

A way to adjust the resistance of the endothelium within the time frame seen in figure 3 may be linked to the ability of endothelial cells to either maintain, or disrupt, junctional
integrity in the face of changes in circumferential stress or active contraction (10).

Typically, in a contracted state, endothelial cells retract away from each other causing the junctional gaps between individual cells to become wider, and the endothelium to become more permeable (9). This retraction is mediated by increased cross-bridge cycling of the actin-myosin complex. Counterbalancing the retraction are the tethering forces provided by adhesive proteins in the adherens junctions. These proteins are linked to the actin-myosin complexes and their function is to maintain a basal level of endothelial barrier function. Endothelial barrier function is directly related to the amount of myosin light chain (MLC) dephosphorylation, accomplished, for example, by molecules such as cAMP. The greater the dephosphorylation, the more effective is the endothelial barrier function. It is possible that the endothelium in the intact arteries experiences changes in MLC dephosphorylation, and hence alterations in the junctional adhesion and intimal permeability, following onset of pulsatile pressure which could explain the initial increase, and subsequent decrease, in arterial permeability. Evidence that endothelial cell permeability may be altered by pulsatile pressure is provided by a study on cultured endothelial cells in which mechanical deformation of the cells by cyclic strain was found to increase oxidative stress (12). Future experiments to investigate the junctional gap size (i.e. by silver nitrate staining), prior to and after changes in the pulsatile pressure, could resolve whether modification of intimal resistance accounts for the lower filtration over time in the intact pulsatile vessels at 60 mmHg baseline pressure.

A transient increase in filtration, similar to that seen after the onset of pulsatile pressure following a steady pressure, was observed when the pulsatile frequency was changed
from 1 to 2 Hz. Since an increase in pulse frequency often happens \textit{in vivo}, the transient burst in filtration is therefore probably a normal physiological event, as opposed to an artifact caused by a sudden onset of pulsatile pressure. When the frequency of pressure pulsatility is changed, the initial transient burst of filtration, seen with the 60-mmHg baseline pressure, is compressed into approximately 2.5 to 15 seconds (compared to 5-30 seconds in the 0-1 Hz frequency change arteries). It is not surprising that in the 1-2 Hz vessels the increased fluid flux occurred over a shorter time period compared with the 60 mmHg 0-1 Hz vessels. Pulsing at twice the frequency but the same amplitude probably serves to move more fluid into the gel matrix at a faster rate.

In both the intact and the de-endothelialized vessels, there was increased filtration compared to that predicted from steady-state measurements. This would indicate that with the onset of a pulsatile pressure internal changes occur in the arterial media, in addition to possible alterations in endothelial permeability, allowing greater filtration to take place. The internal wall modifications could involve either reorganization of the medial structures to allow more passage of fluid through the wall, or a local variation in the internal wall pressure gradient. The lack of any observable difference in fluid influx between the intact and de-endothelialized vessels soon after onset of pulsatile pressure suggests that either the endothelial resistance is reduced during this time, or it is counteracted by altered pressure gradients throughout the artery wall. If the phenomenon seen is due to altered pressure gradients, then the pressure gradients involved would have to be greater in the intact vessels than in the de-endothelialized vessels to cause an equal amount of filtration. Alternatively, the pulsatile pressure could induce the endothelium to
produce substances such as nitric oxide that may alter the configuration, and hence the permeability of the arterial media, possibly by relaxing the smooth muscle cells.

Contemplating the role of the endothelium in large blood vessels, the differences in the filtration responses at longer periods following onset of pulsatile pressure, between the de-endothelialized and the intact vessels, might provide insight into the disease processes associated with inflammation and endothelial dysfunction. For example, if an artery with some endothelial damage were exposed to a change in pulsatile frequency, increased fluid flux could drag higher numbers of macromolecules into the artery wall and allow them to percolate interstitial regions formerly closed to fluid transfer. For example, during the initial period of high filtration more spaces may open up in the medial “ground substance,” (11) or mucopolysacharide gels, allowing access of water and other molecules. Among the transported species could be types of molecules that damage connective tissues and extracellular matrix, such as oxidized LDL, thereby increasing and continuing the inflammatory process. In addition, if the wall ground substance saturates and dampens the excessive convection, then the macromolecules could accumulate beneath the damaged endothelium. Thus a damaged or leaking endothelial barrier might allow large fluxes of lipoproteins and inflammatory species to be convectively driven by pulsatile pressure into the wall so as to overwhelm wall clearance mechanisms. On the other hand, in an intact vessel, although the initial onset of a pulsatile frequency change would cause increased fluid flux, and hence increased macromolecular flux into the artery wall, the endothelium would then act as a dynamic barrier adjusting to different
frequencies and pressures, controlling any subsequent increases in convective fluid and macromolecular fluxes into the artery wall.

A previous study supports the idea that changes in arterial pressure waveforms may affect macromolecular influx as well as fluid influx (7). Chesler and Enyinna (7) investigated particle deposition in \textit{ex vivo} arteries under steady, pulsatile and oscillatory pressure regimes with and without flow. Microspheres deposited in porcine carotid arteries under these regimes were imaged using immunofluorescence and confocal microscopy. Under no flow conditions, pressure waveforms were found to significantly affect the number of 200 nm particles accumulating in the arterial intima. In particular, it was found that a time-varying pressure profile enhanced the amount of particle deposition as compared to a steady pressure at the same mean physiological pressure. The transient filtration spurts observed after changes in pulsatile pressure in the present study are consistent with the increased microsphere accumulation in the subintimal spaces found by Chesler and Enyinna (7). Further experiments testing the role of the endothelium in regulating macromolecular intimal deposition following changes in the arterial pressure waveform could provide insight into the major problem of arterial restenosis following stent placement since this procedure always damages the endothelium.
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References


Table 1: 60 mmHg Baseline Pressure.

Slopes of cumulative filtration volume versus time ($10^{-5} (\text{cm}^3/\text{s})$).

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<td>I. Intact Pulsatile</td>
<td>20.0</td>
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<td>II. De-endothelialized Pulsatile</td>
<td>15.6 (III)</td>
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<tr>
<td>III. De-endothelialized Steady-state*</td>
<td>2.9 (II)</td>
<td>2.7 (II)</td>
</tr>
</tbody>
</table>

*time equivalent for steady-state

A number (roman numeric) after a value indicates a significant difference between that slope value and the slope value corresponding to the numeric (for example, II is significantly different from III above). Intact Pulsatile calculated from data in (1).
Table 2: 80 mmHg Baseline Pressure.

Slopes of cumulative filtration volume versus time (10^{-5}(cm^3)/s)).

<table>
<thead>
<tr>
<th></th>
<th>5-30 Pulses</th>
<th>50-150 Pulses</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Intact Pulsatile</td>
<td>9.0</td>
<td>7.3</td>
</tr>
<tr>
<td>II. De-endothelialized Pulsatile</td>
<td>9.0(III)</td>
<td>9.0(III)</td>
</tr>
<tr>
<td>III. De-endothelialized Steady-state*</td>
<td>4.9(II)</td>
<td>4.1(II)</td>
</tr>
</tbody>
</table>

*Time equivalent for steady-state

A number (roman numeric) after a value indicates a significant difference between that slope value and the slope value corresponding to the numeric (for example, III is significantly different from II at 5-30 pulses). Intact Pulsatile calculated from data in (1).
Figure Legends

Figure 1: Diagram showing the experimental apparatus including the Optical Coherence Tomographer (OCT) (Modified from (1)). Data collected consists of: videotape of bubble position over time and pressure transducer readout files, time-coordinated with graphics files of the OCT a-scans.

Figure 2: Cumulative Volumes at 60-mmHg baseline pressure. Volumes are measures of fluid filtered from the artery lumen into the artery wall. These values are average volumes measured from bubble displacement (from before and after a pulse train) minus volume displacement accounted for by residual distension ($V_{res}$). Steady state volume represents the predicted value of transmural fluid filtered. X-axis represents integrated duration of the pulse trains, such that 5 pulses are one five-pulse train, 10 pulses are two five pulse trains, etc. Error bars are SE of the mean value associated with each point. Trendline ($R^2 = 0.96$) is over values 50-150 of the De-endothelialized vessels. Diamonds represent De-endothelialized pulsatile, triangles represent De-endothelialized steady state, and boxes represent Intact pulsatile (1) volumes.

Figure 3: Cumulative Volumes at 80-mmHg baseline pressure. Trendlines are for the De-endothelialized pulsatile values (lower trendline, $R^2 = 0.98$, slope = 0.00009 cm$^3$/sec, y-intercept = 0.0012 cm$^3$) and Intact pulsatile values (1) 90 through 150 (upper trendline, $R^2 = 0.99$, slope = 0.00007 cm$^3$/sec, y-intercept = 0.0099 cm$^3$). Diamonds represent De-
endothelialized pulsatile, triangles represent De-endothelialized steady state, and boxes represent Intact pulsatile (1) volumes.

Figure 4: Cumulative Volumes at 60-mmHg baseline pressure with pulsatile frequency changed from one to two Hz. Steady state volume represents the predicted value of transmural fluid filtered over the same time as the 1-2 Hz vessels. Diamonds represent 1-2 Hz Pulsatile vessels, triangles represent equivalent steady-state values for the same time as the 1-2 Hz vessels, and boxes represent Intact pulsatile (1Hz) volumes (1).
Figure 1

Figure 2 Vcumulative 60 mmHg De-endothelialized Vessel

- De-endothelialized Pulsatile n = 5
- De-endothelialized Steady-State n = 5
- Intact Pulsatile n = 6
Figure 3: Vcumulative 80 mmHg De-endothelialized Vessel

- **De-endothelialized Pulsatile n = 4**
- **De-endothelialized Steady-State n = 4**
- **Intact Pulsatile n = 5**
Figure 4: Vcumulative, Frequency Change

- 1-2 Hz Frequency Change n = 5
- 1-2 Hz Equivalent Steady-State n = 5
- 0-1 Hz Frequency Change n = 6

Volume cm$^3$

Time (Number of Pulses)