Structural adaptation increases predicted perfusion capacity after vessel obstruction in arteriolar arcade network of pig skeletal muscle

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Gruionu, Gabriel, James B. Hoying, Lucian G. Gruionu, M. Harold Laughlin, and Timothy W. Secomb. Structural adaptation increases predicted perfusion capacity after vessel obstruction in arteriolar arcade network of pig skeletal muscle. Am J Physiol Heart Circ Physiol 288: H2778–H2784, 2005. First published January 28, 2005; doi:10.1152/ajpheart.00917.2004.—Arteriolar arcades provide alternate pathways for blood flow after obstruction of arteries or arterioles such as occurs in stroke and coronary and peripheral vascular disease. When obstruction is prolonged, remaining vessels adjust their diameters chronically in response to altered hemodynamic and metabolic conditions. Here, the effectiveness of arcades in maintaining perfusion both immediately following obstruction and after structural adaptation was examined. Morphometric data from a vascular casting of the pig triceps brachii muscle and published data were used to develop a computational model for the hemodynamics and structural adaptation of the arcade network between two feed artery branches, FA1 and FA2. The predicted total flow to capillaries (QTA) in the region initially supplied by FA2 decreased to 26% of the normal value immediately after FA2 obstruction but was restored to 78% of the normal value after adaptation. After obstruction of 1–10 randomly selected arcade segments, QTA was on average 18% higher in the structural adaptation between two feed artery or of one or more interior segments of the arteriolar network topology. The second goal was to investigate structural adaptation incorporating responses to hemodynamic stimuli (wall shear stress and intra-vascular pressure) and metabolic stimuli including conducted responses. However, the ability of structural adaptation after vessel obstruction to restore perfusion to the region of tissue served by a network of AAs has not previously been assessed using such theoretical models.

The first goal of the present study was to determine the morphometric and topological characteristics of the arteriolar arcade network supplied by two feed arteries of the medial head of pig TBM. Vascular casting was used to determine the vascular network topology. The second goal was to investigate the functional role of the arcades during normal and altered blood supply conditions including the effects of structural adaptation. A theoretical model for network blood flow and structural adaptation (25) was used to simulate the changes in hemodynamic variables resulting from the obstruction of one feed artery or of one or more interior segments of the arteriolar arcade network before and after structural adaptation of the regional blood flow affected by the interruption of blood flow.

The presence of arcade structures in skeletal muscle was noted first in the 19th century by Spalteholz and Cohnheim (37). Since then, arcade network topologies have been described in skeletal muscles of different species including cat biceps femoris, gastrocnemius, soleus, and sartorius muscles (11, 18, 33), rat spinotrapezius muscle (6, 7, 37), rat gracilis muscle (31), and rat cremaster muscle (35). In pigs, the presence of arcades was noted in the triceps brachii muscle (TBM; Ref. 8). Early observations suggested that arcades play an important role during arterial obstruction that would cause death of tissue in the absence of arcades (37). In healthy tissues, the arcades contribute to pressure equalization over the entire organ (17, 37). In a ladderlike representation of the rat cremaster muscle vasculature, the pressure to distal arterioles is more uniform when arcades between upstream arterioles are present (14). More recent experimental (3) and theoretical (20) studies described the hemodynamics of the arcade network of rat gracilis muscle and showed that there is only a small pressure drop and heterogeneous flow in the arcade network.

Blood vessels are capable of structural changes in diameter in response to chronic changes of several local stimuli including wall shear stress (12, 34, 36), intravascular pressure or circumferential stress (2, 10, 16, 30), metabolic state (13, 26), and conducted responses (5, 29). Interruption of blood supply can cause large changes in hemodynamic and metabolic conditions and thereby stimulate structural adaptation of the remaining vessels (9, 27, 32). The role of circumferential stress in remodeling of arcade arterioles (AAs) has been examined theoretically (20–22, 30, 31). Pries et al. (23–25) developed a theoretical model for structural adaptation incorporating responses to hemodynamic stimuli (wall shear stress and intra-vascular pressure) and metabolic stimuli including conducted responses. However, the ability of structural adaptation after vessel obstruction to restore perfusion to the region of tissue served by a network of AAs has not previously been assessed using such theoretical models.

The first goal of the present study was to determine the morphometric and topological characteristics of the arteriolar arcade network supplied by two feed arteries of the medial head of pig TBM. Vascular casting was used to determine the vascular network topology. The second goal was to investigate the functional role of the arcades during normal and altered blood supply conditions including the effects of structural adaptation. A theoretical model for network blood flow and structural adaptation (25) was used to simulate the changes in hemodynamic variables resulting from the obstruction of one feed artery or of one or more interior segments of the arteriolar arcade network before and after structural adaptation of the arterial system. The model predictions were compared with previously published experimental data (31) to validate the model and to investigate the effect of structural adaptation on perfusion of muscle.

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network. Such obstructions of one or more vessels can occur during ischemic events caused by atherosclerotic plaque eruption in stroke or coronary and peripheral vascular disease.

MATERIALS AND METHODS

Vascular casting. This study used one adult (7 mo of age; 34.1 kg body wt) Yucatan miniature swine (Charles River). The animal protocols associated with this study were approved by the University of Missouri Animal Care and Use Committee and were carried out in accordance with the Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (US Government). The heart, lungs, and other vital organs were collected for related cardiovascular studies. After the surgery for organ collection, the TBM tissue remained ischemic for 30 min, which allowed the vascular smooth muscle of the arteries to relax. A polyethylene-200 catheter was inserted at the origin of the extrathoracic segment of the left axillary artery. Physiological saline (200 ml of 0.9% NaCl in 0.01 M phosphate, pH 7.3 at 37°C) was used to flush the vasculature of the forelimb free of blood. The artery was perfused with 27 ml of Microfil silicone rubber injection compound (Flow-Tek) with a viscosity of 120 cP at a perfusion pressure of 90 mmHg (measured with a manometer attached to the injection syringe). After perfusion, the artery was ligated at the site of perfusion to prevent backflow of Microfil compound, and the entire left forelimb was placed flat at 4°C for 24 h to allow the compound to harden. The next day, the medial head of the triceps (weight, 34 g) was carefully dissected and dehydrated in a series of ethanol concentrations (25, 50, 70, 80, 95, and 100%) for 24-h time periods. The muscle was then put in 100% methyl salicylate to clear the tissue. The cleared muscles were transilluminated with a Fiber-Lite high-intensity illuminator. The images of the vascular structures were captured with a high-resolution, black-and-white video camera (Sony) attached to a stereo microscope (Olympus America; Melville, NY). From the camera, the images were transferred to a personal computer using NIH Image software (Scion). The morphometric measurements were performed with SigmaScan software (Statistical Solutions; Saugus, MA).

Previous observations showed that an average of 34 feed arteries supply the medial head of the Yucatan miniature swine triceps muscle (8). The arterial network inside the muscle is a combination of feed artery trees with AAs between their branches. Study of the entire arterioles (TAs) and capillaries. Several other AAs, which are referred to as arteriolar outflows (AOs), branch off the AAs in the observed network and connect to other AAs outside the field of view.

Theoretical model for hemodynamics: A theoretical model was used to estimate the hemodynamic parameters (intravascular pressure, segment flow rate, and wall shear stress) both in the observed AA network (Fig. 1B) and when the network was separated into two tree-type structures (two-tree network, Fig. 1C). The model representation of the network was based on the measured morphometric data (Table 1). For mathematical calculations, the network was represented as a series of cylindrical segments. Nodes were defined as the bifurcation points of the AA segments, the origin points of the feed arteries, and the endpoints of the outflows (TAs and AOs; Fig. 1, B and C).

![Image](http://ajpheart.physiology.org/10.2302/AJP1005-2477.png)
STRUCTURAL ADAPTATION OF ARCADES

Table 1. Morphometric characteristics of the arcade network

<table>
<thead>
<tr>
<th>Feed Arteries</th>
<th>Interior Segments</th>
<th>Transverse Arterioles</th>
<th>Arterial Outflows</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>2</td>
<td>79</td>
<td>58</td>
</tr>
<tr>
<td>Diameter, μm</td>
<td>81 (79–83)</td>
<td>28.8 (12–78)</td>
<td>19.2 (10–45)</td>
</tr>
<tr>
<td>Length, μm</td>
<td>642 (453–831)</td>
<td>585.8 (72–2,134)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means; ranges are shown in parentheses.

length and diameter. The inflows and outflows at node i must sum to zero, giving

\[(P_i - P_{i-1})(R_{i-1} + R_i) + (P_i - P_{i+1})R_i + (P_i - P_i)/R_{iA} = 0\] (1)

A corresponding equation holds at nodes where three AA segments meet. The resulting system of linear equations was solved to obtain the pressures \(P_i\) at each node. The wall shear stress and the mean pressure in segment \(i\) were then calculated using \(\tau_{w,i} = D_i(P_i - P_{i-1})/(4L_i)\) and \(P_{iA} = (P_i + P_{i+1})/2\). Typical viscosity and discharge hematocrit values for vessels in the diameter range of 10–30 μm were then calculated using \(\eta = 3 cP\) and \(HD = 0.4\), respectively (25).

Structural adaptation model. A mathematical formulation based on a previously developed model of structural adaptation (25) was used to describe the structural diameter changes of AA segments. The model is based on the assumption that the diameter of each segment varies with time in response to the local hemodynamic and metabolic stimuli that it experiences. The hemodynamic stimuli considered are wall shear stress and intravascular pressure. A metabolic stimulus is assumed to be generated if flow in a given segment falls below a given level. Effects of upstream conduction of metabolic stimuli along vessel walls are included.

In mathematical terms, with each time step Δt, the diameter \(D_i\) of AA segment \(i\) changes by an amount

\[\Delta D_i = T^{-1} \times S_{\text{int},i} \times D_i \times \Delta t\] (2)

where \(T\) (in days) is a characteristic time scale for structural changes. \(S_{\text{int},i}\) is a dimensionless quantity that represents the combined effects of local stimuli causing structural changes in the diameter of segment \(i\). In the present model, it was assumed that

\[S_{\text{int},i} = \log \tau_{w,i} - \log \tau_w(P_{in}) + k_m \log (Q_{in}/(Q_{in} + 1)) + k_i \left[ S_{c,i}/(S_{c,i} + S_0) \right] + k_b (3)

where the first two terms represent the hemodynamic stimuli: \(\tau_{w,i}\) is the wall shear stress, resulting from blood flow, and \(P_{in}\) is the mean intravascular pressure, which generates circumferential stress. The function \(\tau_w(P_{in})\) is increasing and sigmoidal in form (25). The combined effect of these two terms is to generate a positive correlation between shear stress and pressure as was observed experimentally (25). The third term in Eq. 3 represents the metabolic response and has the effect of causing diameters to increase in segments with very low flow that would otherwise be unable to meet local tissue oxygen demand. The constants in this term are \(k_m\) (the metabolic stimulus constant), \(Q_{in}\) (reference flow blood), and \(HD\) (the discharge hematocrit). The parameter \(Q_{in}\) defines the threshold level of flow. An increasingly strong metabolic growth stimulus is generated if \(Q_{in}/HD\) falls below this level. The fourth term in Eq. 3 represents the conducted response, i.e., the effect of cumulated upstream conduction of the metabolic signal along the vessel wall. \(S_0\) is the reference sum, \(k_i\) is the conducted stimulus constant, and \(S_{c,i}\) is the sum of the conducted stimuli. To compute \(S_{c,i}\), it was assumed that the conducted stimulus is generated in each segment in proportion to the local metabolic stimulus and propagates upstream, decaying exponentially with distance along the vessel. Parameter values describing the metabolic and conducted responses were obtained or estimated from previous studies (25), as follows: metabolic response parameters were \(k_m = 0.81\), \(HD = 0.4\), and \(Q_{in} = 40 \text{ nl/min}\); and conducted response parameters were \(k_i = 2.93\), \(L = 1,000 \mu m\), and \(S_0 = 20\). The last term, \(k_b\), is a constant parameter called the baseline remodeling tendency. It is estimated by varying its value in simulations of the intact case until the predicted total volume of the arcade network matches the intravascular volume estimated from the measured diameters.

Computer codes were written in Matlab language (MathWorks; Natick, MA). For a given distribution of segment diameters, pressures, blood flow rates, and shear stresses for each node and segment were estimated by solving the system of equations (Eq. 1). The resulting total stimulus (\(S_{\text{int}}\)) was then computed according to Eq. 3. Updated AA diameters at the next time step were obtained using Eq. 2. Time steps \(\Delta t = 0.1\) day were used. To determine the steady-state equilibrium diameter, the simulation was continued until the diameters reached an equilibrium state (\(\Delta D \sim 0\)). Simulations were performed for both the arcade network and the two-tree network. The two-tree network was obtained by removing the segments with the lowest blood flow values (determined from hemodynamics calculations) in the arcade network.

Three cases were considered in simulations of the arcade and the two-tree networks, including the intact network, obstruction of FA2, and obstruction of randomly selected AAs. First, the intact network was allowed to reach an equilibrium state according to the adaptation model to provide an appropriate baseline for comparison with simulations in which one or more segments were obstructed. The baseline remodeling tendency \(k_b\) and the conducted stimulus \(S_c\) in the AO vessels were estimated by minimizing the mean squared differences between the measured and the simulated diameters for the intact networks. The resulting values were \(k_b = 0.04\) and \(S_c = 2.7\) in the AOs, and the root mean square difference between measured and fitted AA diameters was then 10.2 μm. This procedure was repeated for the two-tree network, in which case \(k_b = 0.03\) was obtained. These parameters were kept constant for simulations of cases with obstructed segments.

The adapted diameters in the intact network were used as the initial conditions for structural adaptation after segment obstruction. In each case, the simulation was continued until a new equilibrium state was reached. To simulate obstruction of FA2, this segment was removed from the network. To simulate the obstruction of AAs, between 1 and 10 randomly chosen interior segments of the network were removed, and the simulations were repeated. This procedure was repeated 100 times for each number of obstructed segments to estimate the statistical variability. In each case, hemodynamic parameters including TA flows and wall shear stresses in arcade segments were computed immediately after obstruction (nonadapted) and after diameters reached a stable equilibrium (adapted), and the total TA flow was calculated. The mean and standard error of the 100 estimates of total TA flow were computed for each number of interrupted segments.

Computer visualization. To illustrate the simulation results, three-dimensional computer visualizations of the network were generated. Vessel diameters were shown at 2× scale for clarity. The grayscale picture of the Microfil compound-perfused network was imported into the Pro/Engineer software package (Parametric Technology; Needham, MA). All bifurcation points and a variable number of points at equal distances along each vessel length were manually recorded from photographs to identify their two-dimensional coordinates. The points were then joined with spline curves representing the vessel centerlines. Each vessel segment was modeled as a blend surface with a circular cross-section whose diameter varies smoothly between the points at which diameter is specified.

RESULTS

Arcade network topology. The preparation allowed visualization of vessels to a depth of ~2 mm within the muscle. Feed arteries enter the medial head of the pig triceps muscle at
multiple points (8) and branch several times within the muscle. The terminal branches supply a dense three-dimensional network of AAs (Fig. 1A). For the purpose of the present study, an arcade network lying between two main feed-artery branches was selected based on its visibility within the available depth of field. A computer graphic representation of this network is shown in Fig. 1B. The two feed artery branches were labeled FA1 and FA2. The arcade network was represented as a set of 143 segments that included 79 AAs, 58 TAs, and 6 AOs. Morphometric data on these segments are given in Table 1.

Intact arcade network. The steady-state distribution of segment diameters and flows after structural adaptation of the intact arcade network is indicated graphically in Fig. 2A. To compare the arcade network with a network that contained only tree structures, a two-tree network (see Fig. 1C) was formed by removing the arcade segments with lowest flow and opposite flow direction at the two nodes (yellow in Fig. 2A). This procedure also defined regions 1 and 2 as the arteriolar trees of FA1 and FA2, respectively (see Fig. 1C). In the intact network, the total TA flow, average shear stress, and average arcade segment diameter were approximately the same in regions 1 and 2 (Fig. 3).

Obstruction of one feed artery. Interruption of blood flow through FA2 caused redistribution of blood flow within the arcade network as indicated in Figs. 2B and 3A (middle bars). In region 1, total TA flow was almost unchanged, but shear stress increased twofold, which reflects the fact that AAs in region 1 also supplied region 2 (Fig. 3, A and B). In region 2, total TA flow decreased to 26% of its normal value (Fig. 3A). Additional hemodynamic changes after simulated structural adaptation are shown in Figs. 2C and 3 (right bars). In response to increased wall shear stress, diameters of segments in the middle region of the arcade network were significantly increased and formed larger conduits to region 2 (see Fig. 2C). Of the four connecting pathways between FA1 and FA2 in the intact network, two pathways became the main conduits, whereas the other two were secondary (see Fig. 2C). On average, region 1 diameters increased by 22%, and region 2 diameters decreased by 7% (Fig. 3C). As a result of these structural changes, total TA flow in region 2 was restored to 78% of its original value (Fig. 3A). In the two-tree network, by contrast, interruption of FA2 would obviously cause complete cessation of flow in region 2, with no change in region 1, and structural adaptation would have no ability to restore flow to region 2.

Obstruction of multiple AAs. Results of obstructing 1–10 interior segments of the arcade network are shown in Fig. 4. Total TA flow decreased with increasing number of blocked segments. This decrease was more rapid in the two-tree network than in the arcade network. Although the two network

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**Fig. 2.** Computer visualizations show diameters and flow rates in the arcade network. A: intact network. B: FA2 blocked, not adapted. C: FA2 blocked, adapted. Flow rates (in nl/min; see color scale, bottom) are shown in color ranging from yellow (lowest) to dark red (highest).
topologies have equal total TA flow when they are intact, the arcade network had on average 18% higher total TA flow than the two-tree network. With structural adaptation, flow was significantly restored in the arcade network but not in the two-tree network. The increase in TA flow with adaptation in the arcade network averaged 16%. Corresponding results are also shown in Fig. 4 for the case of FA2 obstruction. The initial effect on TA flow was similar to the effect of obstructing seven arcade segments, but the restoration of flow with structural adaptation was more effective, and the final total TA flow was similar to that obtained when four arcade segments were obstructed.

**DISCUSSION**

Two challenges in studying the arcade network topology of the pig triceps muscle are the large number of vessels and the three-dimensional nature of the network. The AAs form a continuous mesh, and discrete modular structures are not identifiable. Because the network is three dimensional, it cannot be characterized as a set of well-defined arcade loops as can be done for thin skeletal muscles or other tissues that have essentially two-dimensional arcade networks (6, 19, 33). Here, we considered a functional unit consisting of the three-dimensional network of AAs connecting two feed arteries on the surface of the muscle. Connections to other AAs were represented by arcade outflows. Similar examples of arcade networks could be seen throughout the muscle vasculature between any two or three feed arteries. The present formulation of the theoretical analysis could be applied to any such network.

The presence of a network of AAs provides a degree of robustness to muscle perfusion when supply vessels are obstructed. In the absence of arcades (i.e., a tree topology), the entire area normally perfused by an obstructed vessel would receive no blood. With arcades present, blood is redirected from intact supplies toward the affected area. In the example considered, the area initially fed by FA2 still received 26% of normal perfusion when FA2 was removed. Similarly, when between 1 and 10 interior segments were blocked, the arcade network delivered on average 18% higher flow to capillaries than the corresponding two-tree network, which had no arcades.

Tissue survival after obstruction of blood supply depends not only on the existence of alternate pathways for blood flow but also on the long-term capacity of the remaining vessels to restore perfusion. The latter requirement is accomplished by collateralization, i.e., structural adaptation in which the vessels forming the alternate pathways remodel outward, thereby allowing them to carry increased flow. This process was simulated here using a previously developed adaptation model for responses to local hemodynamic and metabolic stimuli (1, 25). In the example considered, structural adaptation in response to long-term blockage of a feed artery led to restoration of perfusion to the affected area to 78% of normal perfusion. Similarly, when interior segments were blocked, structural adaptation resulted in an increase in perfusion that averaged 16%. This occurred because remaining segments experience increased wall shear stress and increased conducted metabolic responses that cause structural increases in diameters. These results suggest that parallel pathways provide not only routes for blood flow but also for upstream information transfer to parent vessels (28). In the two-tree structure without arcades,
no alternate pathways are available to feed segments downstream of obstructions, and so no such benefit from structural adaptation is possible.

Structural adaptation of AAs only was simulated in the present model. In reality, TAs and other downstream vessels may also adapt structurally in response to altered flow conditions. Similarly, the arcade outflows may also adapt, but this was not included in the model. Inclusion of such responses would probably enhance the ability of the network to adjust to obstruction of feed arteries or AAs. Therefore, the above estimates of the degree to which structural adaptation can restore perfusion via AAs are likely to be conservative. In this study, the measured and simulated diameters of AAs refer to fully dilated conditions. Use of dilated diameters in the simulations is justified because conditions that lead to structural diameter increase (high shear stress, high metabolic demand) also lead to vasodilation. However, the relationship between vascular tone and structural remodeling is not well understood.

Direct observations of microvascular structures within the pig TBM over extended periods are experimentally difficult. In the present study, theoretical simulations using a previously developed model were therefore used to predict quantitatively the ability of arcade structures in combination with structural adaptation to maintain and restore flow after obstruction of small arteries or arterioles in skeletal muscle. Specifically, the model predicts that the existing arcade structure is capable of maintaining a relatively low level of flow (26% of normal in this example) to a region whose arterial supply is cut, and that subsequent structural adaptation leads to a restoration of flow to a level that approaches normal flow (here, 78% of normal). These model predictions could be tested by measuring changes in perfusion over time after controlled obstructions of blood flow in corresponding experimental systems.

In summary, our observations show that the pig TBM contains a dense, three-dimensional network of AAs. The functional role of this system was investigated using a theoretical analysis of hemodynamics and structural adaptation of vessel diameters in a part of the network that connects two feed artery branches. The presence of arcades was shown to give a degree of robustness to muscle perfusion when supply vessels are obstructed by providing alternative flow pathways. This effect is enhanced by structural adaptation, which further restores perfusion by the formation of enlarged collateral flow pathways.

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