Marriage of resistance and conduit arteries breeds critical limb ischemia

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Coats, Paul, and Roger Wadsworth. Marriage of resistance and conduit arteries breeds critical limb ischemia. Am J Physiol Heart Circ Physiol 288: H1044–H1050, 2005; doi:10.1152/ajpheart.00773.2004.—Atherosclerosis in a major leg artery leads to impaired blood supply, which normally progresses to critical limb ischemia. Atherosclerosis produces substantial alterations of structure and endothelial function in the large conduit arteries. Pressure unloading and ischemia in the distal vasculature bring about alterations in microvascular function. Resistance arteries undergo significant wall thinning and changes in their contractile regulation. Optimization of large artery dimensions by the small arteries through flow-mediated vasodilation is impaired. Angiogenesis is stimulated, which can result in the formation of major collateral feeder vessels in addition to small nutritive blood vessels. However, angiogenesis can also contribute to instability of atherosclerotic plaques, which ultimately leads to further deterioration in blood supply. Surgical bypass grafting to restore blood supply to the distal leg generates a sudden increase of pressure in the weakened resistance vasculature, leading to uncontrolled changes in capillary hydrostatic pressure, extravasation of fluid, and tissue edema. This review aims to highlight the importance of the resistance vasculature in critical limb ischemia and the interdependence of pathophysiological changes in the large conduit and small resistance arteries. The major unresolved question is why the physiological mechanisms that regulate vascular structure and function ultimately break down, leading to circulatory failure within the distal limb.

In time, chronic ischemia, as a primary consequence of macrovascular disease, results in a number of structural and functional adaptations to the microcirculation (20). Arteriolar vasodilation, and consequent reduction of peripheral vascular resistance, is a primary compensatory response to ischemia (51). Peripheral arterioles in CLI patients are relatively insensitive to vasodilator stimuli compared with those in control subjects, thus demonstrating that arterioles in CLI patients are maximally dilated (28). McEwan and Ledingham (55) proposed that compensatory vasodilation results in arteriolar dysfunction due to the chronic exposure of the arterioles to vasoconstricting factors. They called this phenomenon “vasomotor paralysis,” which has been identified as a contributing factor in edema formation in CLI (51). In many CLI patients, elevation of the feet results in capillary collapse and “pallor” of the distal limb. Conversely, when the patients stand, blood drains unimpeded into the capillary beds of the lower limb. The lower limbs become suffused with blood, observed as “rubor” (51). Uncontrolled orthostatic-dependent increases in hydrostatic pressure within the distal portion of the limb are likely to contribute to the development of edema. Edema formation is a paradoxical finding, because CLI, by definition, is a state of hypopressure and hypoperfusion. Nonetheless, in ~70% of patients with CLI, edema is confined to the distal portion of the affected leg (44, 45). More than 90% of CLI patients undergo angioplasty and/or vascular reconstruction within 1 yr of diagnosis (67). In a large number of CLI patients, bypass grafts that are fully patent are not associated with normal blood perfusion to the peripheries, with a significant number of these patients forming postoperative edema. Consequently, many of these patients must undergo amputation of the affected leg (33, 62, 72). After these surgical interventions, postoperative leg edema can develop as a consequence of the reintroduction of systemic blood pressure to the distal portion of the limb. Edema formation in CLI further potentiates tissue metabolic insufficiency, inasmuch as edema compresses the delicate capillaries and impairs diffusion of nutrients to the surrounding tissues (55). The orthostatic-dependent changes in lower limb blood perfusion described by Lowe (51) and the phenomenon of postoperative edema after revascularization demonstrate a circulatory inadequacy to autoregulate blood hydrostatic pressure and would favor the development of edema in CLI patients. The ability to autoregulate blood flow to the capillary beds is largely dependent on myogenic mechanisms of vascular tone,

CRITICAL LIMB ISCHEMIA (CLI) represents ischemia of such severity as to compromise lower limb function and endanger survival of the affected leg(s). It occurs when the supply of blood to the capillary beds is less than that required to sustain surrounding tissue viability. CLI has an annual incidence of 1 in 1,000 population and is associated with a significant increase in morbidity and reduced life expectancy, and patients generally have a poor prognosis (40). The primary cause of CLI is atherosclerotic lesions within the large communicating arteries. The resistance to blood flow produced by these lesions results in a critical reduction in perfusion pressure to the distal portion of the affected leg(s). Consequently, blood flow to the distal part of the leg(s) results in ischemia and, consequently, ischemic pain, tissue necrosis, and gangrene (51).
an intrinsic property of resistance arteries (56). Thus, despite the disease process in CLI originating at the level of the large communicating arteries, the subsequent downstream chronic ischemia produces structural and functional adaptations within the small resistance arteries. Paradoxically, rectification of the large artery disease, the primary cause of the condition, through surgical intervention is consequently compromised by the secondary small artery disease.

PATHOPHYSIOLOGICAL CHANGES IN CONDUIT ARTERIES

Artery structure. Remodeling of artery structure in accordance with changing physiological demands has been extensively studied in animal models and has also been demonstrated in human leg arteries. However, almost two-thirds of the population in late middle age has evidence of atherosclerotic disease of the femoral artery (49), and normal artery wall structure cannot be maintained in plaque regions. In healthy subjects, leg exercise increased the diameter and reduced intima-media thickness of the femoral artery. The mechanism probably involves increased blood flow through the femoral artery, initiating vasodilation and outward remodeling of the femoral artery wall (30). In contrast, areas of atherosclerotic plaque show inward remodeling and intimal hypertrophy. In patients with more advanced disease, exercise will be limited, and the resulting loss of exercise-induced outward arterial remodeling will exacerbate the deterioration in artery structure caused by atherosclerotic disease.

Endothelium. Endothelial function is impaired in patients with peripheral artery disease. In a study of patients with identified significant disease affecting a major leg artery, flow-mediated vasodilation in the brachial artery was substantially reduced compared with the matched control group (9, 89). Because endothelial responsiveness was impaired in a vascular bed distant from the most severe atherosclerotic plaques, these data are consistent with the concept of atherosclerosis as a systemic disease with consequences that are widespread throughout the vasculature. Endothelial dysfunction was found to correlate with plasma concentrations of the inflammatory markers C-reactive protein, fibrinogen, soluble intercellular adhesion molecule-1, and soluble vascular cell adhesion molecule-1 (9, 10). These data demonstrate a significant degree of inflammation in patients with leg ischemia. One mechanism through which inflammation may contribute to endothelial dysfunction is production of oxygen radicals that destroy nitric oxide (NO) and have cytotoxic actions. Consistent with this view, it has been found that administration of L-arginine to hypercholesterolemic subjects improved flow-mediated vasodilation and normalized the plasma level of thiobarbituric acid-reactive substances (a measure of oxidative stress) (43).

Shear forces acting on the endothelium increase expression of NO synthase and superoxide dismutase, which, along with other endothelial mediators, are important in the vasculoprotective effects of exercise. Exercise was found to improve endothelium-dependent relaxation in middle-aged subjects, including hypercholesterolemic subjects who were treated with lipid-lowering agents (87, 88). However, exercise training produced less aortic wall stress in older than in younger subjects (17, 18), and this may be one reason for deteriorating endothelial function with increasing age.

Flow-mediated vasodilation has been demonstrated in the human femoral artery during reactive hyperemia. Reactive hyperemia is caused by a downstream vasodilation and increased blood flow, which releases vasodilator factors from the endothelium of the feeder artery, with consequent relaxation. In healthy human subjects, the femoral artery remained dilated for ≥3 min after the shear force on the femoral artery endothelium had returned to baseline (60). Additional mechanisms for communication along the length of an artery include endothelial cell-to-cell communication (31). These studies demonstrate a powerful modulation of the distributing arteries by the microvasculature; however, it is not clear how the endothelium brings about long-lasting changes in artery structure.

ANGIOGENESIS IN LIMB ISCHEMIA

The vascular network has a remarkable degree of plasticity and the capacity for significant restructuring through angiogenesis, arteriogenesis, and arterial remodeling. Angiogenesis (capillary sprouting) and arteriogenesis (expansion of preexisting collaterals) are physiological responses to increased demand for blood supply and are stimulated by factors such as tissue hypoxia and alterations in blood flow. Angiogenesis involves the coordinated migration, proliferation, assembly, and maturation of endothelial cells, smooth muscle cells, and other cell types and is under the control of several growth factors, of which the most extensively studied are vascular endothelial growth factor (VEGF), fibroblast growth factor, platelet-derived growth factor, and angiopoietin.

VEGF is produced by the vascular endothelium and other cells of the artery wall and causes endothelial cell proliferation and endothelial tube formation. The effects of VEGF and NO are intertwined. Thus VEGF stimulates NO formation by endothelial cells, and the angiogenic actions of VEGF appear to be mediated by NO (90). Moreover, NO induces VEGF mRNA and protein synthesis in cultured smooth muscle cells (34). The improvement in limb blood flow and capillary density induced by endothelial NO synthase gene administration was abolished by anti-VEGF antibody (61).

In a mouse model of leg ischemia (femoral artery ligation), the development of new capillaries was impaired in atherosclerotic compared with control mice. Blood flow restoration was also slower in the atherosclerotic animals and correlated with capillary density. In the atherosclerotic mice, infiltration of T cells, which were positive for VEGF, was reduced, and thus VEGF was reduced in the ischemic limbs (24). Recovery of blood flow after femoral artery ligation was also delayed in another mouse model of hyperlipidemia compared with wild-type mice. Administration of hepatocyte growth factor restored capillary density and blood flow to the level observed in mice with normal lipid profiles but did not accelerate angiogenesis in wild-type mice (57). Similar improvements in capillary density and blood flow were found in a rat hindlimb ischemia model (79).

Angiogenesis can occur in various locations, resulting in beneficial or detrimental consequences. Improved blood flow may result from increased density of nutritive arteries in the ischemic limb tissue or from formation of bypass channels that divert blood around an obstruction (24, 59, 79). In contrast, angiogenesis within atherosclerotic plaque tissue is considered to be detrimental, because new vessels in this location are highly susceptible to rupture, and intraplaque hemorrhage is
one of the key factors leading to plaque deterioration and instability (8, 41). VEGF is localized to these intraplaque vessels (8, 41), and in a cholesterol-fed rabbit model, administration of recombinant VEGF increased atherosclerotic plaque area (16). Thus it is possible that angiogenesis may limit or, in contrast, promote the progression of CLI.

In patients with peripheral artery disease, elevated plasma VEGF was reduced to normal on revascularization (54, 70), suggesting that angiogenesis is stimulated during limb ischemia. Attempts have been made to administer VEGF by gene therapy to patients with leg ischemia. In preliminary trials, ankle blood pressure and collateral vessel formation were improved (6, 71). However, in a randomized double-blind study, no significant difference in walking time or ankle blood pressure was found between VEGF gene-treated and control groups (71). This negative clinical result compared with the positive results in animal studies may indicate that the dose of VEGF delivered in this particular trial was insufficient, that VEGF needs to be augmented by other growth factors, or that detrimental angiogenesis in plaque areas is a limiting factor.

PATHOPHYSIOLOGICAL CHANGES IN RESISTANCE ARTERIES

Resistance arteries with an internal diameter of ~150 μm contribute significantly to total peripheral resistance and basal vascular tone (58). The fundamental function of resistance-sized arteries is control of blood flow to the capillary beds, which maintains an optimal pressure-flow relation, which in turn is essential for gas and nutrient exchange from blood to tissue. This is partly achieved by a putative pressure-sensing mechanism, which stimulates a myogenic response in the vascular smooth muscle (25, 56). The ability of a resistance artery to withstand pressure-dependent distension is the sum of two intrinsic components: active myogenic tone and passive structural properties of the artery.

Pressure-dependent vascular wall stress and, to a lesser extent, shear stress are recognized as the major determinants of vascular wall structure (12, 46, 69). There have been few in vitro studies on the effect of reducing environmental stimuli, such as blood pressure and blood flow, on vascular structure and function and certainly none in human subjects over an extended period. Studies have demonstrated arteriolar structural and functional plasticity as a product of intraluminal pressure and flow dynamics (26, 70). This is most evident in animal models of hindlimb unloading, where the animal is suspended by the tail for a period of days and blood volume is unloaded from the hindlimb, inducing a cephalic fluid shift and postural muscle unloading (26). These animals manifest many of the adaptations that are found in CLI, including postural muscle atrophy, diminished capacity to elevate vascular resistance in the skeletal muscle microvasculature within the limb, uncontrolled orthostatic-dependent changes in limb volume, and reduced aerobic muscle capacity (26). Studies of skeletal muscle arterioles from these animals indicate that at least part of the inability to control vascular resistance results from a blunting of myogenic autoregulation (26). Significantly, the microvascular effects of hindlimb unloading have also been reported to be readily reversible after normal orientation, with restoration of vascular structure and function, including pressure-dependent myogenic reactivity (52).

Hypertension has long been associated with structural changes in small resistance arteries characterized by hypertrophic or eutrophic inward remodeling (3–5, 37). There are, however, only a few studies of the effects of low blood pressure (hypotension) and low blood flow on resistance vascular structure (19, 20, 70). In these studies, reversal of the hypertensive stimuli reversed hypertension-associated structural adaptations (12), low flow resulted in outward hypertrophic remodeling (69, 70), and low blood pressure resulted in a reduced media-to-lumen ratio (21). The structural changes observed in resistance arteries from patients with CLI, a hypotensive state, are essentially the opposite of those observed in hypertension, thus reaffirming the association between blood pressure and vascular structure. Pressure-dependent mechanical forces are known to influence vascular structure. The mechanisms involved are unclear, but mechanical forces have been shown to upregulate vascular smooth muscle DNA synthesis and cell growth (69, 86). Moreover, many of the structural adaptations to blood flow and pressure have been observed to be endothelium dependent via the release of endothelium-derived mitogens, such as angiotensin II and endothelin, or the enhancement of endothelial cell adhesion molecules and subsequent activation of mitogenic growth factors such as platelet-derived growth factor (48, 65, 72, 74). These studies have focused on hypertension and/or intimal hyperplasia; however, if these factors are upregulated as a consequence of increased blood pressure-increased shear stress, then it is plausible that they are downregulated in low blood pressure-low shear stress, such as CLI. Vascular structure is closely correlated with function (58). Resistance artery responses to agonist- and pressure-dependent (myogenic) stimuli are much greater in hypertensive than in normotensive models (29, 35, 80).

CLI, a hypotensive-hyperperfusion state, is correlated with morphological changes that are the opposite of those observed in hypertension, i.e., decreased wall thickness, decreased cross-sectional area, and decreased wall-to-lumen ratio compared with controls (20). Furthermore, in CLI, the changes in vascular wall composition parallel changes in vascular wall mechanics. Specifically, incremental distensibility, wall stress, wall strain, and tangential elastic moduli are altered, favoring increased compliance and reduced ability to withstand pressure-dependent dilation. Clearly, the resistance in the distal portion of a chronically ischemic limb has neither the structural nor the functional properties of a resistance artery. Therefore, it is not surprising that, after reintroduction of higher pressure, edema forms in the affected part of the leg. These observations are important, because they provide an explanation for postoperative edema development after the return of systemic perfusion pressures following revascularization, which can result in graft failure and poor wound healing, which in turn may accelerate the requirement for amputation in CLI patients.

Endothelium. CLI is associated with hemostasis and microthrombosis within the capillaries plus edema, all of which have been attributed to endothelial dysfunction (32). The vascular endothelium has several key physiological functions, including modulation of vascular tone, antithrombogenic barrier, and control of vascular permeability. There have been no in-depth studies on the effect of chronic ischemia on vascular endothelium of small resistance arteries. However, the effect of stroke and ischemia-reperfusion injury on endothelial structure and function...
function has been studied in the cerebral resistance vasculature (1, 27, 52). In these studies, ischemia has been associated with endothelial cell swelling, increased endothelial permeability, and, subsequently, edema formation (1, 53). These changes have been associated with an increase in release of VEGF (1, 72, 82). Moreover, after ischemia, the vascular endothelium in the cerebral vasculature has been shown to take on a proinflammatory phenotype, resulting in increased expression of adhesion molecules and release of numerous vasoactive and proinflammatory mediators, including prostaglandins, leukotrienes, cytokines, and platelet-activating factor (50, 81). Similarly, microthrombosis, endothelial swelling, and edema have been reported as being potential causes of microcirculatory blood flow abnormalities in CLI (15, 22, 36).

Diabetes and hypertension have been associated with resistance artery endothelial dysfunction (48, 76). In CLI the pathophysiological effects are thought to be a consequence of a complex set of endothelium-dependent events that evoke an inappropriate activation of microvascular-dependent flow and deposition of microthrombi within the microvascular beds (32, 51). Central to the ischemia-dependent activation of microvascular-dependent flow is release of endothelium-derived relaxing factor (EDRF). However, in CLI, where diabetes and hypertension are prevalent, there is likely a degree of endothelial trauma that may be responsible for increased free radical production, inappropriate platelet activation, leukocyte adhesion, and, consequently, microthrombi formation. However, our own studies have shown that the endothelium in resistance arteries isolated from ischemic regions is intact and displays vasodilator function comparable to that of control tissues (20). This alone is a surprising observation, inasmuch as CLI patients are often diabetic and/or hypertensive; thus it may be that what appears to be preserved endothelial function may, in fact, be compensatory. Further studies are required to elucidate this point.

Ischemia is, in part, compensated by the release of EDRFs (51), which results in arteriolar vasodilation, increased blood flow, and cessation of ischemia. The constant activation of the vasodilator compensation mechanism via release of the EDRFs, factors known to inhibit protein synthesis, over a prolonged period must have some effect on artery structure. This may be advantageous in the early stages of CLI; however, over time, this mechanism may have an indirect detrimental effect on artery function because of the inhibition of protein synthesis. This, combined with the reduction in blood flow and pressure-dependent stimuli that helps maintain arterial wall structure, may explain the atrophic changes observed in the arteries isolated from the ischemic vascular beds of patients with CLI (21).

Oxygen radicals. The generation of phagocyte-derived oxygen free radicals, which is detrimental to vascular endothelial function, is a key initiating step in the atherogenic process in large arteries. However, there is no evidence for similar atherogenic processes in the small resistance arteries. There is growing evidence that reactive oxygen species (ROS), such as hydrogen peroxide and superoxide, play an important role in intracellular cell signaling (7, 42, 83). The NAD(P)H oxidase system is a principal generator of intracellular ROS in vascular smooth muscle cell signaling (38, 75). The capacity of vascular smooth muscle NAD(P)H oxidase to generate ROS is much lower than that of phagocytes, and generation of ROS appears to be mainly intracellular (39). In fact, ROS via NAD(P)H oxidase has been shown to modulate myogenic tone in resistance-sized arteries (63). These findings are important, inas-
much as they clearly show that ROS are not detrimental to vascular function and do, in fact, play a crucial role in modulating fundamental physiological vascular performance, namely, pressure-dependent myogenic tone.

**Perspectives**

CLI is a common manifestation of atherosclerosis, characterized by progressive deterioration of the limb circulation. Studies of conduit and resistance arteries from patients with CLI have shown marked physiological changes in advanced disease. Studies in animal models have elucidated mechanisms that maintain healthy structure and function of large and small arteries and their interdependence. The two recognized compensatory mechanisms recruited in CLI are compensatory vasodilation and formation of a collateral circulation. These mechanisms are presumably adequate in the initial stages of peripheral vascular disease, yet in patients who progress to CLI, physiological compensation is no longer effective (Fig. 1). A full understanding of why these compensatory mechanisms fail is likely to require integration of knowledge of the physiology of resistance arteries, conduit arteries, and new vessel formation through angiogenesis.

Patients with CLI will, wherever possible, undergo a surgical revascularization procedure such as a bypass graft. Limb revascularization has a high success rate as judged by the restoration of blood supply to the distal part of the leg. However, despite this success in the immediate postoperative period, the intervention fails because of edema formation and consequent infection and breakdown of tissue. Edema formation is ubiquitously observed after revascularization in CLI. Studies of resistance artery function in CLI have identified impairment of pressure-dependent function, which will favor uncontrolled increase in capillary hydrostatic pressure, leading to edema. Thus it is important to understand the mechanisms of the physiological changes in resistance arteries during ischemia and pressure unloading to find a therapeutic intervention to prevent revascularization edema. Ultimately, a treatment strategy needs to be directed at both the conduit and the resistance arteries.

**REFERENCES**


