The role of shear stress in the pathogenesis of atherosclerosis

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Although the pathobiology of atherosclerosis is a complex multifactorial process, blood flow-induced shear stress has emerged as an essential feature of atherogenesis. This fluid drag force acting on the vessel wall is mechanotransduced into a biochemical signal that results in changes in vascular behavior. Maintenance of a physiologic, laminar shear stress is known to be crucial for normal vascular functioning, which includes the regulation of vascular caliber as well as inhibition of proliferation, thrombosis and inflammation of the vessel wall. Thus, shear stress is atheroprotective. It is also recognized that disturbed or oscillatory flows near arterial bifurcations, branch ostia and curvatures are associated with atheroma formation. Additionally, vascular endothelium has been shown to have different behavioral responses to altered flow patterns both at the molecular and cellular levels and these reactions are proposed to promote atherosclerosis in synergy with other well-defined systemic risk factors. Nonlaminar flow promotes changes to endothelial gene expression, cytoskeletal arrangement, wound repair, leukocyte adhesion as well as to the vasoreactive, oxidative and inflammatory states of the artery wall. Disturbed shear stress also influences the site selectivity of atherosclerotic plaque formation as well as its associated vessel wall remodeling, which can affect plaque vulnerability, stent restenosis and smooth muscle cell intimal hyperplasia in venous bypass grafts. Thus, shear stress is critically important in regulating the atheroprotective, normal physiology as well as the pathobiology and dysfunction of the vessel wall through complex molecular mechanisms that promote atherogenesis.

Laboratory Investigation (2005) 85, 9–23, advance online publication, 29 November 2004; doi:10.1038/labinvest.3700215

Keywords: shear stress; atherosclerosis; endothelium; smooth muscle cells; nitric oxide; cytoskeleton; mechanotransduction

The parallel frictional drag force of shear stress is one of the important blood flow-induced mechanical stresses acting on the vessel wall, which also includes the perpendicular force of blood pressure and the cyclic stretch of pulsatile flow. Shear stress is a biomechanical force that is determined by blood flow, vessel geometry and fluid viscosity that is computationally estimated using fluid dynamics models and is expressed in units of dynes/cm².¹ Shear stress forces are imposed directly on the endothelium and modulate endothelial structure and function through the activities of local mechanotransduction mechanisms with the ultimate activation of shear stress response promoter elements/transcription factors, that modulate endothelial gene expression. Several molecules, structures and processes have been implicated in mechanotransduc-

tion of shear stress into specific biochemical signals and/or activated intracellular signaling pathways, resulting in modulation of cellular structure and function. The precise molecular mechanisms remain unknown; however, much new knowledge has been published to begin to understand how the cell senses shear stress, what signaling pathways are regulated by these molecular sensors and which genes are regulated by the downstream elements of these pathways. Indeed, due to normal laminar flow, shear stress is a critical factor in maintaining normal physiologic vascular function including thromboresistance, barrier function and vascular homeostasis. However, the pathogenesis of atherosclerosis is a complex multifactorial process of vascular wall injury and atheroma formation due to dynamic local and systemic factors² (Figure 1). Shear stress, especially when blood flow is disturbed and/or is nonlaminar, plays an important role in the pathogenesis of the atherosclerotic plaque, especially where flow conditions are disturbed with low or oscillatory shear stress. The combination of altered arterial hemodynamics around curvatures, arterial branch ostia and bifurcations,¹ where secondary

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Received and revised 20 October 2004; accepted 21 October 2004; published online 29 November 2004

Figure 1 A model of atherogenesis at sites of decreased hemodynamic shear stress. Note that the decreased shear stress affects cellular morphology and numerous cell functions, several of which are atherogenic. In the presence of systemic risk factors, some of which are listed, there is an increased likelihood of atherosclerotic plaque formation at these sites, which once formed, further disrupts flow and promotes growth of the fibroinflammatory lipid plaque.

flows occur, and systemic risk factors promote atherosclerotic lesion initiation, progression and ultimately, development of complicated plaques. For example, atherogenesis is promoted by decreased shear stress (<5 dynes/cm²) because it is associated with reduction in several vascular wall functions including endothelial nitric oxide synthase (eNOS) production, vasodilatation and endothelial cell repair. These are coupled with increases in reactive oxygen species (ROS), endothelial permeability to lipoproteins, leukocyte adhesion, apoptosis, smooth muscle cell proliferation and collagen deposition.³ In addition, many risk factors linked to cardiovascular disease such as hypertension, smoking, hyperlipidemia, hyperhomocysteinemia and diabetes mellitus have a direct impact on endothelial cell function⁴ and impair flow-mediated vasodilatation in a progressive and additive fashion.⁵ This is significant because endothelial-dependent vasomotion has been identified as an independent predictor of cardiac events.⁶ Fluid shear stress does play an important role in blood vessel formation, especially in angiogenesis, arteriogenesis and in aneurysm formation. These topics are beyond the scope of this review.

Vascular shear stress

Shear stress is expressed in the case of laminar flow where the profile of blood velocity is parabolic as

$$\tau = 4\mu Q/\pi r^3$$

where μ is the viscosity, Q is the flow rate and r is the vessel radius. Vascular shear stress of large

conduit arteries typically varies between 5 and 20 dynes/cm²; however, significant instantaneous values range from negative measures to nearly 40 dynes/cm² during states of increased cardiac output.¹ This continuous exposure to a physiologic range of shear stress, especially in straight sections of the artery exposed to unidirectional laminar flow, promotes the establishment of important physiologic characteristics of the artery wall promoting an anti-inflammatory, antithrombotic, anticoagulative, profibrinolytic and antihypertrophic state. Shear is also integrally involved in flow-mediated vasodilatation as a means to normalize mechanical forces and thus helps to maintain normal endothelial function. Flow conditions in the artery are specified by the Reynolds number (Re), which is a measure of the stability of flow:

$$Re = 4\rho Q/\pi\mu D(\text{g/cm}^3)$$

where ρ is the density of blood, D is the vessel diameter, μ is the blood viscosity and Q is the flow rate. This represents a ratio of inertial fluid momentum to viscous frictional forces. Thus, at very low flow rates, Re below 1 g/cm³, viscous forces dominate over inertial forces and flow follows the geometry of flow channels. Secondary flows (vortices and other nonaxial flow phenomena) arise at greater Re when viscous forces and inertial forces are comparable. Secondary flows develop at arterial bends and bifurcations. Transition to turbulence, where lateral motion of the blood becomes fully randomized, occurs at Re of 500 to 1500 g/cm³ and is dependent on vascular geometries, the presence or absence of lumen stenosis and on flow pulsatility.⁷

Transient turbulence has been shown to occur in the human aorta at peak systole, during heavy dynamic exercise in much of the central arterial system and distal to arterial luminal stenosis and aneurysms.

Thus, the endothelium is exposed to diverse flow patterns: laminar flow, which is the steady tangential drag force noted above; oscillatory flow, where time-averaged fluctuations in shear stress are very low or zero due to forward-reverse flow cycles and disrupted flows, which exhibit regions of flow separation, recirculation and reattachment that are associated with temporal and spatial shear gradients.¹ Vascular smooth muscle cells (SMC) are also directly or indirectly responsive to shear stress resulting in regulation of cell proliferation, migration and differentiation between contractile and secretory phenotypes.⁸ The vascular wall is continuously fine-tuning its activities in response to vascular shear stress. However, significant changes and/or disruption of laminar flow impart changes in endothelial cell behavior, which alters the biology of the endothelial monolayer and subsequently the susceptibility of conduit vessels to atherosclerotic disease.

In studies by Brad Berk's group on how vascular remodeling maintains vessel lumen diameter and shear stress, they found that shear stress is differentially regulated among several inbred rat strains. This suggested that genetic determinants were important in regulating shear stress in response to changes in blood flow.⁹ Linder's group and Berk's group have also carried out vascular remodeling experiments, which support the notion that in mice vascular remodeling is strain dependent.^{10,11}

Endothelial and vascular SMC interactions

Activated, proliferating vascular SMC play an essential role during atherogenesis. These cells contribute to intimal hyperplasia that forms the bulk of the plaque, to expression of growth factors, cytokines, and proinflammatory mediators such as monocyte chemoattractant protein-1 (MCP-1) and vascular cell adhesion molecule (VCAM) and to synthesis of matrix molecules some of which are important for retaining lipoproteins.¹² Much of the endothelial-smooth muscle communication is through paracrine pathways; the best studied being nitric oxide (NO) regulation of vasodilatation. Although the internal elastic lamina separates medial vascular SMC from the abluminal endothelium, numerous membrane pores (2–7 μm) permit direct communication between the endothelial cells and vascular SMC, potentially through myoepithelial bridges.^{13,14} Interactions between these cell types are important for proper vessel wall function and both have been shown to be responsive to shear stress. For example, laminar shear stress inhibits not only endothelial but also vascular SMC prolifera-

tion.^{15,16} Chiu *et al*¹⁷ demonstrated that in a modified coculture system exposed to the physiologic 12-dynes/cm² fluid flow, vascular SMC tended to orient themselves perpendicular to the direction of flow in contrast to endothelial cells, which are known to orient themselves parallel to flow. Also, shear stress less than 2 dynes/cm² had no effect on vascular SMC orientation. Importantly, coculture under static conditions induced leukocyte adhesion proteins GRO- α and MCP-1 expression while application of shear stress attenuated this response. This observation suggests that shear stress acts through the endothelium to modulate SMC gene expression imparting an atheroprotective phenotype on the vessel wall. The uptake of acylated-low-density lipoproteins (Ac-LDL) is decreased in endothelial-SMC cocultures exposed to laminar shear stress relative to static cultures. Interestingly, shear stress increased uptake of unaltered LDL, suggesting that uptake of oxidized LDL is favored under low flow conditions,¹⁸ similar to that seen at those sites predicted at risk of atheroma formation. Nonuniform shear stress has been shown to influence the *in vivo* density of SMC in the vessel wall through a graded suppression of mitogenic activity at higher shear levels. The level of shear stress was inversely correlated with intimal SMC density, whereas exposure to laminar flow did not influence cell density.¹⁹ Altogether, these studies uphold the premise that shear stress acts on vascular SMC in part through endothelial interactions to modulate SMC gene expression, uptake of atherogenic lipoproteins and proliferation as a means of suppressing atheroma formation.

Mechanotransduction of shear stress

There appears to be several possible mechanisms by which endothelial cells sense stress and act as a shear transducer including those mediated by gene expression and those not mediated by gene expression. Following the initial mechanosensing, cell surfaces and cell membranes may be deformed, ions may be translocated, local biochemical responses may be activated, downstream intracellular signaling pathways may be activated and endothelial genes may then be expressed, all to modulate shear-induced endothelial function as well as important shear-induced alterations in endothelial cell morphology (Figure 2).

An early response to shear stress is the influx of Ca²⁺ leading to increased cytosolic Ca²⁺, which may lead to numerous changes in intracellular activation of molecules and production of bioactive agents such as NO.¹¹ The membrane potential modulates the driving force for Ca²⁺ transmembrane fluxes since endothelial cells lack voltage-dependent Ca²⁺ channels. Ion channels that are mechanosensitive have been identified and characterized.²⁰ Laminar shear stress promotes upregulation of IKCa1

Figure 2 A schematic diagram of the pathways by which shear stress may regulate atherogenesis. The pathogenesis of the atherosclerotic plaque is modulated by shear stress regulation of atheroprotective and atherogenic influences, by systemic risk factors and by genetic influences that affect both multiple cellular processes in the wall and the plaque as well as the response of the vessel wall to shear stress itself.

transcription, resulting in increased intermediate-conductance Ca^{2+} -activated K^+ channel (IK_{Ca}) activity, which results in hyperpolarization, and membrane-driven Ca^{2+} influx, which may be atheroprotective because of a likely increased synthesis of vasodilator and antithrombotic factors.²¹ Flow-activated ion currents including the hyperpolarizing current carried by inward rectifying K^+ channels and a depolarizing current due in part to outward rectifying Cl^- channels show different sensitivities to high and to low shear stress and to oscillatory shear stress.²² The endothelial cytoskeleton is also proposed to play a central role in the mechanotransduction of shear stress with direct participation of each of the three main structural cytoskeletal fibers as well as indirect involvement of cytoskeletal-associated structures such as focal adhesions and adherens junctions.^{23–25} Conformational changes in the cell cytoskeleton in response to shear forces may promote mechanotransduction, as may conformational changes in the cell–cell and the cell–substratum adhesion complexes. At the substratum, integrin–matrix interactions are also implicated in transduction primarily by activation of downstream pathways that are implicated in both anti- and pro-atherogenic activities.²⁶ The glycocalyx²⁷ is postulated to be disrupted by shear stress leading to modifications of ion channels and membrane receptors. Other factors and structures have been studied including dystrophin,²⁸ caveolae,²⁹ PECAM-1,³⁰ gap junctions³¹ as well as many other ancillary

structural and signaling proteins. Rapid, shear-induced activation of Ras, a small G protein,³² may lead to activation of mitogenic signaling pathways through extracellular signal-regulated kinase (ERK) and Jun kinase (JNK). Exposure of endothelial cells to shear stress results in NO due to the dissociation of eNOS from caveolin-1.³² Chronic laminar shear stress induces translocation of caveolin-1 from the Golgi to the plasma membrane, increases caveolin formation at the luminal side and changes ERK and Akt activation, reducing the former and enhancing the latter.³³ Resnick *et al*¹ reviewed the data that suggest that induction of laminar shear stress results in three patterns of gene expression immediate followed by decrease to static levels less immediate maintaining the altered levels of expression for several hours and delayed induction several hours after onset of shear stress. However, it is still unknown how shear stress ultimately results in the numerous transcriptional changes mediated by shear stress responsive promoter elements (SSREs) that bind transcription factors, which are also activated by shear stress. It must be noted that the exact mechanisms of converting a mechanical stress into a biochemical signal is thus not well understood and requires further study. The hope is that both shear stress receptors and shear stress responsive genes will be useful as biomarkers of atherosclerotic disease and as targets for therapies of atherosclerosis.

Vascular biologic activity regulated by shear stress

Expression patterns of many endothelial gene products that are recognized as being up- or down-regulated in response to laminar and/or disturbed flows have been examined either in individual candidate gene studies or in large DNA array-based genomic analyses.^{34,35} A subset of these shear regulated factors are secreted by endothelial cells and may be grouped into functional classes that include vasoactive compounds, growth factors, coagulation/fibrinolysis/complement factors and extracellular matrix (ECM)/ECM modifying factors (Table 1).^{36–56} That many of these secreted products fall into functional classes should not be surprising as disturbed shear stress is likely to alter endothelial cell biology in a directed fashion, as a means to adapt to the new environmental conditions. Although for some gene products such as NO and tumor growth factor β (TGF β), the response to shear stress is apparent, for others, such as platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and angiotensin converting enzyme (ACE), the impact of shear stress is less clear.⁵⁷ The majority of studies on candidate genes and their products that were found to be responsive to shear were performed using *in vitro* endothelial monolayers exposed to various flow conditions in

Table 1 Atherogenic/atheroprotective factors modulated by shear stress

	References
<i>Vasoactive compounds</i>	
Angiotensin converting enzyme (ACE)	36
NO—endothelial nitric oxide synthase (eNOS)	37
NO—induced nitric oxide synthase (iNOS)	38
Prostacyclin	39
C-type natriuretic peptide	40
Adrenomedullin	41
Endothelin-1	42
<i>Growth factors</i>	
Platelet-derived growth factor B (PDGFB)	43
Epidermal growth factor (EGF)	44
basic Fibroblast Growth Factor (bFGF)	45
Melanoma growth-stimulatory activity/ growth-regulated gene—chemokine (GRO)	46
Granulocyte monocyte-colony stimulating factor (GM-CSF)	47
Insulin-like growth factor binding protein (IGFBP)	55
<i>Coagulation/fibrinolysis/complement</i>	
Thrombomodulin	48
Tissue factor	49
tissue Plasminogen Activator (tPA)	50
Protease-activated receptor-1—thrombin receptor (PAR-1)	51
Properdin—complement factor	52
<i>ECM/ECM degradation enzymes</i>	
Collagen XII	53
Matrix metaloprotease-9 (MMP-9)	54
<i>Inflammation</i>	
Monocyte chemoattractant protein (MCP-1)	69
Vascular cell adhesion molecule (VCAM-1)	68
Intercellular adhesion molecule (ICAM-1)	67
E-selectin	70
Bone morphogenic protein (BMP-4)	75
<i>Others</i>	
Extracellular superoxide dismutase (ecSOD)	117
Sterol regulatory element binding protein (SREBP)	170
Platelet/endothelial cell adhesion molecule (PECAM-1)	29

milieus quite different from the *in vivo* vessel. An example of enhancing *in vitro* experimental models is the work of Michael Gimbrone's group who designed an *in vitro* dynamic flow system to reproduce arterial shear stress waveforms applied on cultured endothelial cells. They used 3D flow analysis based on actual aortic artery bifurcation geometries and blood flow profiles to characterize an athero-prone and an atheroprotective waveform, which were applied to the endothelial cells in culture to analyze gene expression using transcription profiling.⁵⁸ Studies of gene expression *in vivo*, under conditions of either uniform or disrupted flow, have appeared^{35,59} confirming some but not all of the large body of *in vitro* data and shedding further light on potentially significant changes in

factor production in regions at risk for developing *in vivo* atheromatous lesions.

Investigators in the field are cautious since gene expression profiles differ among different studies; however, among classes of genes there are similarities in global gene expression. For example, laminar shear stress promotes expression of anti-inflammatory, antiproliferative, antiapoptotic and antioxidative genes. There are differences in the findings between various *in vitro* studies, which may reflect different models, use of cells from different species or derived from different locations, and different experimental design and sampling times. These differences have not yet been pursued with any rigor. In addition, flow preconditioning of cultured endothelial cells is considered important since these cells will change their phenotype, at least to some extent, adopting phenotypes that may not completely reflect those of *in vivo* endothelial cells. Thus, designing experiments that allow for the establishment of a hemodynamic environment prior to studying how alterations in shear stress alter cell morphology and function is a very useful experimental strategy.

Inflammation

Shear stress plays an important role in regulating the inflammatory processes that initiate and enhance the growth of the fibroinflammatory lipid plaque. Shear stress appears to heavily influence vascular inflammation through modification of endothelial gene expression to a proatherogenic profile. Many groups have utilized microarray analyses of endothelium under conditions of laminar vs disturbed shear stress to suggest that nonlaminar flow can result in gene expression of proinflammatory molecules in the vascular wall.^{60–62} Indeed, recent work has shown that both shear stress and cyclic strain in combination may better predict sites of proatherogenic gene expression.⁶³ Analysis of vascular regions prone to atherogenesis due to disturbed blood flow *in vivo* have revealed that altered shear stress can prime the endothelium to respond to proatherogenic stimuli through upregulation of the proinflammatory transcription factor NF- κ B.^{35,59} Laminar shear stress promotes an antiinflammatory and antioxidative expression profile, acting through promoter elements identified as SSREs,⁶⁰ antioxidant response elements (ARE)⁶⁴ and PPAR γ -response elements.⁶⁵ Shear stress also induces phosphorylation of Ser-727 of the transcription factor STAT3 via the ERK 1/2 pathway, which inhibits Tyr-705 phosphorylation in STAT3. This results in a reduced translocation of STAT3 into the nucleus and this attenuation of STAT3 activation reduces inflammatory responses through reduced cytokine stimulation.⁶⁶ However, the interaction between shear stress and inflammation appears to be more complex given that laminar shear stress can also modulate

proinflammatory cytokine (TNF α)-dependent transcription in endothelial cells.⁶⁷ Thus, a fine balance exists between the expression of atheroprotective and proatherogenic genes mediated by levels of laminar shear stress and decreased shear, respectively.

Low shear stress influences adhesion of leukocytes to activated endothelium through enhanced expression of adhesion molecules such as ICAM-1,⁶⁷ VCAM-1,⁶⁸ MCP-1⁶⁹ and E-selectin.⁷⁰ In addition, laminar flows have been found to antagonize the ability of the proinflammatory cytokine, TNF α , to recruit leukocytes and induce expression of vascular endothelial growth factor (VEGF)-1 and E-selectin,^{67,71} reinforcing the balance sought between the pro- and anti-inflammatory influences of oscillatory and laminar shear stress, respectively. Mediation of this antagonism may involve inhibition of MAP kinase activation (eg JNK) as well as association of TRAF-2 with TNFR-1.⁷² Interestingly, laminar shear stress also inhibited vascular SMC-dependent promotion of endothelial adhesion molecule expression in coculture under static conditions, a process requiring close communication between the two cell types.⁷³ Bone morphogenic protein-4 (BMP-4), a member of the TGF β family of cytokines, has recently been shown to be upregulated in endothelium over lipid-filled atheroma as well as in those cells exposed to oscillatory shear stress.⁷⁴ Elevated levels of BMP-4 were also correlated with increased ICAM-1 expression through an NF- κ B-dependent mechanism. It is worth noting that BMP-4 was previously identified in calcified atherosclerotic plaques⁷⁵ and calcific heart valves,⁷⁶ however, its specific role in shear-stress-dependent leukocyte adhesion is unknown.

Nitric oxide

NO is a central mediator in vascular biology and physiologic flow is the primary stimulus for continuous NO production by the endothelium.⁷⁷ NO synthase (NOS) catalyzes the NADPH-dependent conversion of L-arginine and O₂ to L-citrulline and the short-lived radical NO. One of the primary functions of NO involves the regulation of vascular caliber to modulate blood flow and normalize endothelial shear stress and cyclic strain.⁷⁸ The molecular mechanisms of NO activity include roles in signaling, free radical scavenging and post-translational protein modification through S-nitrosylation.⁷⁹ NO is atheroprotective through enhanced vascular relaxation as well as through inhibition of platelet activation and aggregation, apoptosis and endothelial-dependent monocyte adhesion.^{80–82} However, NO is not necessary to suppress endothelial activation chronically; rather it counterbalances factors that arise, which promote proatherogenic changes in endothelial cell behavior.⁸³ Attenuation of NO is one of the earliest biochemical changes preceding endothelial dysfunction, which is an

important concept highlighted by a recent meta-analysis showing that common genetic variations in the eNOS gene, and presumably NO availability, is an important risk factor in atherosclerosis and ischemic heart disease.⁸⁴

Endothelial NOS is regulated by biomechanical stimuli and multiple biochemical factors, including acetylcholine,⁸⁵ H₂O₂,⁸⁶ estrogen,⁸⁷ bradykinin,⁸⁸ sphingosine-1-phosphate,⁸⁹ VEGF,⁹⁰ TNF α ,⁹¹ as well as shear stress and flow-related aerobic exercise.⁹² In diseased vessels, the endothelium overlying atherosclerotic plaques shows a decrease in eNOS mRNA and protein expression,⁹³ which correlates well with plaques occurring and growing in regions of disturbed blood flow where there is impaired endothelial-dependent vasorelaxation.^{93,94}

The complexity of eNOS activity involves multiple levels of regulation, not the least of which is gene expression. NF- κ B is a transcription factor activated in regions of disturbed flow and is predicted to mediate proinflammatory gene expression in response to shear stress.^{35,59} This phenomenon is believed to be associated with a decrease in eNOS activity. Interestingly, Davis *et al*³⁷ show that under conditions of laminar flow, NF- κ B binds to the eNOS promoter and transiently upregulates eNOS transcription, followed by a prolonged increase in the stability of eNOS mRNA.⁹⁵ Given that NO inhibits NF- κ B activation,⁹⁶ it is suggested that laminar shear stress may provoke a negative feedback loop with shear stress inducing eNOS expression through NF- κ B, which is followed by increased NO production and subsequent NO-dependent decreases in NF- κ B activity. Factors that interfere with NO activity, such as ROS, that are produced in regions of low shear stress, could potentially lead to unchecked elevations in NF- κ B-dependent, proinflammatory gene expression.³⁷

The mechanism of eNOS activation appears to involve its subcellular location, an association in multiprotein complexes, as well as targeted tyrosine phosphorylations. Endothelial NOS was shown to be associated with caveolae and is inhibited through its interaction with caveolin-1.⁹⁷ Although eNOS has also been found to be associated with other subcellular structures such as the Golgi apparatus and the mitochondrion, the functional significance of these interactions is unclear.⁹⁸ Multiple protein partners of eNOS have been identified in which some promote eNOS activity such as Ca²⁺/CaM,⁹⁹ hsp90,¹⁰⁰ dynamin-2,¹⁰¹ porin¹⁰² and guanylate cyclase¹⁰³ and some suppress eNOS activity such as caveolin-1,⁹⁷ G-protein coupled receptors,¹⁰⁴ and NO-interacting protein (NOSIP).¹⁰⁵ Recent evidence also demonstrates that eNOS interacts with and is inhibited by the transmembrane intercellular adhesion molecule, PECAM-1, a protein postulated to act in the mechanotransduction pathway.¹⁰⁶ This interaction is disrupted over seconds in response to acute increases in shear stress and correlates with a parallel increase in eNOS activity. However, the

interaction is not perturbed in response to shear stress with a low temporal gradient. Acute changes in shear stress are also associated with tyrosine phosphorylation of PECAM-1,¹⁰⁷ an event required for the flow-dependent activation of ERK,³⁰ a MAP kinase.

Production of eNOS as a result of shear is biphasic. Initially, there is a Ca^{2+} /CaM-dependent disruption of eNOS' interaction with caveolin-1, resulting in the production of NO secondary to acute changes in shear stress. Then there is a more Ca^{2+} -independent, slow production of NO in response to steady laminar flows.⁹⁹ The precise mechanism of steady NO production is not entirely clear, yet binding of Hsp90 to eNOS¹⁰⁰ and protein kinase A (PKA)-dependent phosphorylation of eNOS S635 (bovine sequence) appear to play a role.¹⁰⁸ Targeted phosphorylation of eNOS on residues S1179, S635 and potentially S617 and S116 (bovine sequences) may also increase enzyme activity in response to shear stress.¹⁰⁹ Additionally, it appears that dephosphorylation of T497 is required for optimal activation of eNOS;¹¹⁰ however, appropriate phosphorylation of T497 is argued to regulate production of NO as opposed to superoxide anions from eNOS.¹¹¹ Numerous kinases have been identified for each site, especially S1179, including PKA,¹¹² Akt,¹¹³ PKG,¹¹⁴ AMP-activated protein kinase (AMPK)¹¹⁰ and CaM-dependent kinase II (CaMKII).¹¹⁵ Laminar shear stress appears to act primarily through Akt and PKA. Supportive roles for the other enzymes have been suggested.¹⁰⁹ A recent observation demonstrated that the catalytic subunit of PKA is associated with eNOS in luminal lamellipodia located near endothelial cell junctions in restricted intracellular locales,¹¹⁶ the existence of which may help to elucidate differential regulation of eNOS by PKA. Shear stress has been shown to activate the vascular endothelial growth factor II receptor (VEGFR2) in a ligand-independent manner, recruiting phosphoinositide 3-kinase (PI_3K) and subsequently activating Akt and eNOS.¹¹⁷ Interestingly, like PECAM-1, VEGFR2 activation is associated with shear stress-dependent tyrosine phosphorylation, in this case known to be src-dependent, over the same time frame of PECAM-1 phosphorylation and eNOS release from caveolae and PECAM-1. However, whether any of these events are linked is still unknown. Exercise training has been shown to induce a shear-dependent increase in c-src activity associated with eNOS and extracellular superoxide dismutase activation.¹¹⁸ Related studies have also shown that shear stress induced by exercise induces enhanced S1179 phosphorylation¹¹⁹ and potentially recruits eNOS containing caveolae to the plasma membrane,²⁹ possibly helping to explain the means by which regular exercise can be atheroprotective. Laminar shear stress has also been shown to increase the S-nitrosylation of proteins such as caspase-3 and thioredoxin,¹²⁰ facilitating the antiapoptotic and antioxidative role of steady shear flows.

It is postulated that oscillatory shear stress, disrupted flows and turbulent flows act on the vascular wall to disrupt these atheroprotective molecular pathways reducing the production of NO and thus changing the biology of the vessel. This results in the production of proinflammatory mediators through NF- κ B-dependent transcription, impaired flow regulated vasorelaxation and reversal of the antiapoptotic, antiproliferative (relevant to vascular SMC) and antioxidative (relevant to LDL) functions of the endothelium. Microparticles, which are membranous vesicles released from activated T lymphocytes with procoagulant and proinflammatory properties, induce endothelial dysfunction by decreasing eNOS expression and overexpressing caveolin-1 in endothelial cells activated during atherogenesis.¹²¹ This observation serves to illustrate that once the atheroprotective role of the endothelium, in part through its eNOS activity, has been compromised, a vicious cycle of vascular wall injury inducing plaque formation and subsequent disruption in blood flow can facilitate atheroma progression and potentially impart vulnerability onto atherosclerotic lesions. An important recent study shows that a single nucleotide polymorphism of the human eNOS gene (-786C/T) renders affected patients at higher risk of coronary heart disease through a mechanism that is the result of an inability for shear stress to maintain basal levels of eNOS expression.¹²²

Reactive oxygen species

The association between ROS production and atheroma formation is well established and involves shear stress. ROS production occurs in many cell types in the vascular wall such as endothelial cells, vascular SMC, adventitial fibroblasts and macrophages.¹²³ There are at least five different enzyme systems contributing to the oxidation state of the vascular wall. These include lysyl oxidase (LOX), responsible for the covalent cross-linking of collagen and elastin,¹²⁴ xanthine oxidase (XO),¹²⁵ monocyte-derived ROS,¹²⁶ uncoupled eNOS¹²⁷ and the NAD(P)H family of oxidases, which are the primary contributors of ROS species in the vasculature.¹²⁸ ROS increase leukocyte adhesion molecule expression,¹²⁹ stimulate SMC proliferation and migration, promote lipid oxidation, upregulate matrix metalloproteinase activity and alter vasomotor activity.¹³⁰

Shear stress modulates the redox state of the vascular wall, in part, by regulating expression of the different oxidase systems involved. The NAD(P)H family of oxidases are structurally different from the well-known phagocytic NAD(P)H oxidases and can be regulated by angiotensin II, through a phospholipase D-dependent process,¹³¹ inflammatory cytokines and shear stress.¹³² Endothelial cells exposed to oscillatory shear stress show a marked increase in NAD(P)H activity and ROS production in contrast to the antioxidative state

induced by laminar flow.¹³² It has been proposed that XO expression may be regulated by NAD(P)H oxidase activity and that XO is actually the primary source of ROS in the vascular wall in response to oscillatory shear stress.¹²⁵ Chronic exposure of endothelial cells to oscillatory shear stress increased monocyte adhesion, which is dependent on NAD(P)H oxidase activity.¹²⁹ Finally, it was recently demonstrated that vascular NAD(P)H oxidase subunit, NOX4, expression increased in response to oscillatory shear stress and was associated with an increase in LDL oxidation relative to that in static cultures.¹³³

XO, expressed by endothelium binds extracellular glycosaminoglycans and was found to be higher in individuals with coronary artery disease compared with normal controls.¹³⁰ eNOS, although classically described as being atheroprotective, can significantly contribute to ROS production if electron transfer within the enzyme becomes uncoupled secondary to loss of either the cofactor 5,6,7,8-tetrahydrobiopterin (BH₄) or the substrate L-arginine, resulting in the transfer of electrons to O₂, forming superoxide.¹²⁷ Interestingly, hypercholesterolemia,¹³⁴ diabetes¹³⁵ and hypertension¹³⁶ have all been associated with eNOS uncoupling, potentially through ROS production, further potentiating the creation of superoxide.

In addition to the upregulation of oxidases, shear stress may promote changes in other antioxidative systems. Intracellular glutathione, providing reduction equivalents, is decreased under nonlaminar flows^{130,137} and the enzyme glutathione peroxidase is upregulated under laminar flow conditions.¹³⁸ Of the three forms of the atheroprotective superoxide dismutase (SOD) found in the vascular wall,¹³⁹ extracellular SOD and Cu/Zn SOD may be stimulated by shear stress indirectly through enhanced eNOS production.^{140,141} Elevated levels of extracellular SOD have even been found in patients with acute coronary syndromes, potentially representing an important protective factor against oxidative stress associated with ischemic events.¹⁴² As stated above, adequate eNOS-dependent NO radical production allows ROS to be continually scavenged. Whether the vascular biology is impacted principally by loss of eNOS activity with an associated rise in ROS vs primarily negative effects associated with elevated ROS is unclear.

Systemic cardiovascular risk factors such as smoking,¹⁴³ hypertension,¹⁴⁴ diabetes¹⁴⁵ and hyperlipidemia,¹⁴⁶ which are known to influence shear stress, are associated with elevated NAD(P)H oxidase activity and increased oxidative stress in the vessel wall. Importantly, angiotensin II receptor antagonists and ACE inhibitors have been shown to reduce NAD(P)H oxidase activity,¹⁴⁷ suggesting a role for the renin-angiotensin system in promoting vascular oxidative stress.¹⁴⁸ Statins also appear to reduce oxidative stress through suppression of NAD(P)H oxidase activity, independently of its role

as an HMG-CoA reductase inhibitor.¹⁴⁹ A role for antioxidants and L-arginine to modulate the proatherogenic effects of turbulent blood flow has been suggested using cell culture and murine models;¹⁵⁰ however, successfully reducing vessel wall oxidative stress with antioxidants in human patient populations has not been as encouraging.¹⁵¹ Although work has focused on the role of oxidative stress in promoting atheroma formation, plaque evolution and vulnerability are also advanced by the redox state of the vasculature, potentially associated with angiogenesis and thus with intraplaque hemorrhage.¹⁵²

Vascular permeability

Disturbed shear stress directly influences endothelial integrity and thus permeability due, in part, to modulation of cytoskeletal contractile function (see below). Regions prone to atherosclerosis have increased uptake by the arterial wall of fluorescent, radiolabeled and chromogenic tracers modeled to represent serum lipoprotein particles.¹⁵³ Disturbed flow may increase particle transit times in susceptible regions¹⁵⁴ and it has even been suggested that the proliferative effects of nonlaminar flows may enhance permeability during mitosis of endothelial cells.¹⁵⁵ In the porcine proximal external iliac artery, a region known for complex blood flow, endothelial permeability to Evans blue dye tagged albumin decreased with increasing time-averaged shear stress in the physiologic range in contrast to the increased permeability observed under oscillatory shear conditions.¹⁵⁶ However, enhanced retention of lipoprotein particles by the extracellular matrix and not increased transport *per se*^{25,154} may promote the site selectivity of atheroma formation.

Cytoskeleton

Shear stress has a profound effect on the endothelial cytoskeleton,^{25,157–159} especially the actin and the microtubule cytoskeletons, which are essential in maintaining the integrity of the vascular endothelium, and regulating endothelial repair following endothelial disruption and/or endothelial denudation due to cell loss.^{157–159} Actin microfilaments are arranged both centrally and peripherally in endothelial cells to form stress fibers¹⁶⁰ and a dense peripheral band,¹⁶¹ respectively. Stress fibers are long bundles of actin filaments containing myosin, tropomyosin and alpha-actinin that can contract and exert tension.¹⁶² They are linked to the plasma membrane at focal adhesion sites where they serve to promote strong attachment to the substratum.²⁴ The dense peripheral band, on the other hand, plays a role in cell-cell adhesion^{161,163,164} and likely acts, at least in part, to regulate endothelial permeability. Partial disassembly of the adherens junction complex is induced by shear stress and is followed by

reassembly that is associated with endothelial shape change and reorganization of F-actin distribution.¹⁶⁴ The distribution and organization of the actin cytoskeleton is altered in response to hemodynamic shear stress. For example, low hemodynamic shear stress favors prominent peripheral microfilaments,¹⁵⁹ while elevated hemodynamic shear stress¹⁶⁵ promotes an increase in central microfilaments. In cells exposed to high shear conditions, the central microfilaments become both thicker and longer, while the dense peripheral band becomes disrupted. These changes are reversible if normal shear is reinstated.¹⁶⁵ Sustained exposure to flow also induces cytoskeletal remodeling that results in orientation of the endothelial cells in the direction of flow.^{166,167} Physiologic shear stress has been shown to be necessary for timely repair of the mechanically injured endothelium.¹⁵⁹ Within *in vivo* mechanical injury models, low flow rates are associated with poor cell-cell contacts and disordered alignment of the cytoskeleton in cells along the wounded endothelium resulting in disturbed repair.¹⁵⁹ Disturbed repair may not only promote atheroma formation through enhanced endothelial permeability but may also lead to the formation of endothelial erosions and fissures that are associated with complicated atherosclerotic lesions and rupture of unstable plaques.¹⁶⁹ In a hypercholesterolemic rabbit model actin microfilaments undergo prominent changes, which initially may promote endothelial-substratum adhesion but eventually results in a reduction of stress fibers and dysfunctional adhesion.¹⁷⁰ An interesting finding linking shear stress, actin microfilament system and cholesterol metabolism is that shear stress activates the Rho-ROCK-LIMK-cofilin pathway, which results in modulation of sterol regulatory element binding proteins (SREBPs) that regulate lipid and cholesterol metabolism.¹⁷¹

Vein grafts and restenosis

Shear stress has been implicated in affecting long-term vein graft patency, following coronary artery bypass procedures and vascular remodeling. Vein graft adaptations following introduction of arterial pressures involve elements of both intimal hyperplasia and expansive remodeling with local shear stress levels determining the balance between the two. Graft failure may be a consequence of enhanced neointimal hyperplasia secondary to vascular SMC proliferation from predominantly vein-derived cells.¹⁷² Interestingly, preferential fibromuscular hyperplasia of the vein intima at the distal anastomosis,¹⁷³ which is likely secondary to disturbed flows, may have a secondary effect on the long-term flow velocity through the body of the graft, tipping the balance toward lower shear stresses and enhanced neointimal hyperplasia. Late restenosis following balloon angioplasty and stent placement is also associated with neointimal hyperplasia,

which is inversely related to the shear stress distribution within the arterial segment, such that low shear stress regions are associated with maximal intimal thickness and high shear regions with minimal thickness.¹⁷⁴ Numerous models have been proposed to explain the changes in shear stress. These include the creation of disturbed shear secondary to the significantly elevated flow velocities,¹⁷⁵ prolonged inflammatory response to the stent promoting vascular SMC proliferation/migration¹⁷⁶ and the stent design itself modulating local shear gradients.¹⁷⁷ It is intriguing to observe that intimal hyperplasia has also been shown to occur in regions of low shear stress proximally and distally to the stent itself,¹⁷⁸ suggesting that the stent may indeed have more far-reaching influences on arterial architecture.

Conclusions

Shear stress is involved in both physiologic and pathophysiologic vascular biology. Modulation of vascular wall behavior in regions of disturbed blood flow in conjunction with the impact of systemic vascular factors such as smoking, hyperlipidemia, hyperglycemia and hypertension promotes a chronic fibroinflammatory response to the resultant arterial injury. This response is associated with a disarming of the atheroprotective defenses encouraged by laminar blood flow. The consequences of this dynamic interaction between shear stress-induced changes and systemic risk factors not only promotes atherosclerosis but also influences the success of those therapeutic modalities used to modify disease progression and clinical outcomes. A major challenge in the field is to integrate the large body of data on shear-dependent endothelial gene expression to identify useful biomarkers for atherosclerosis, to design appropriate approaches to prevent atherogenesis and to provide useful therapeutic interventions for atherosclerosis, especially complicated fibroinflammatory lipid plaques.

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