Brief Review

Laminar Shear Stress
Mechanisms by Which Endothelial Cells Transduce an Atheroprotective Force

Oren Traub, Bradford C. Berk

Abstract—Mechanical forces are important modulators of cellular function in many tissues and are particularly important in the cardiovascular system. The endothelium, by virtue of its unique location in the vessel wall, responds rapidly and sensitively to the mechanical conditions created by blood flow and the cardiac cycle. In this study, we examine data which suggest that steady laminar shear stress stimulates cellular responses that are essential for endothelial cell function and are atheroprotective. We explore the ability of shear stress to modulate atherogenesis via its effects on endothelial-mediated alterations in coagulation, leukocyte and monocyte migration, smooth muscle growth, lipoprotein uptake and metabolism, and endothelial cell survival. We also propose a model of signal transduction for the endothelial cell response to shear stress including possible mechanotransducers (integrins, caveolae, ion channels, and G proteins), intermediate signaling molecules (c-Src, ras, Raf, protein kinase C) and the mitogen activated protein kinases (ERK1/2, JNK, p38, BMK-1), and effector molecules (nitric oxide). The endothelial cell response to shear stress may also provide a mechanism by which risk factors such as hypertension, diabetes, hypercholesterolemia, and sedentary lifestyle act to promote atherosclerosis. (Arterioscler Thromb Vasc Biol. 1998;18:677-685.)

Numerous studies suggest that normal functioning of the endothelium is critical in limiting the development of atherosclerosis, as illustrated by the correlation between risk factors for atherosclerosis (smoking, high cholesterol, high homocysteine, decreased estrogen, increasing age, and hypertension) and endothelial dysfunction.1 Endothelial cells play a critical role in vascular homeostasis by performing many functions. They sense and integrate hemodynamic and hormonal stimuli and effect alterations in vascular function through the secretion of various mediator proteins and molecules.2 As a result of these properties, endothelial cells modulate biological processes related to the blood vessel wall, including regulation of the permeability of plasma lipoproteins, adhesion of leukocytes, and release of prothrombotic and antithrombotic factors, growth factors, and vasoactive substances.3 Impairment of these endothelial cell–mediated processes has been postulated to play a central role in the pathogenesis of atherosclerosis.4

Just as other tissues have developed mechanisms to detect changes in the physiological conditions to which they are exposed, endothelial cells respond not only to humoral factors in the circulation but also to the mechanical conditions created by blood flow and the cardiac cycle.4 As a result of their unique location, endothelial cells experience three primary mechanical forces: pressure, created by the hydrostatic forces of blood within the blood vessel; circumferential stretch or tension, created as a result of defined intercellular connections between the endothelial cells that exert longitudinal forces on the cell during vasomotion; and shear stress, the dragging frictional force created by blood flow. Of these forces, shear stress appears to be a particularly important hemodynamic force because it stimulates the release of vasoactive substances and changes gene expression, cell metabolism, and cell morphology.4

The nature and magnitude of shear stress plays an important role in long-term maintenance of the structure and function of the blood vessel. The nature of shear stress experienced by endothelial cells is a function of blood flow patterns throughout the vasculature generated by the cardiac cycle. In “linear” areas of the vasculature, blood flows in ordered laminar patterns in a pulsatile fashion dependent on the cardiac cycle, and endothelial cells experience pulsatile shear stress with fluctuations in magnitude that yields a mean positive shear stress. This flow pattern should be distinguished from the flow pattern that is often used in experimental preparations and that generates a steady positive shear stress, being temporally and spatially uniform. While steady shear stress generally stimulates many of the same endothelial cell responses as pulsatile stress, there are some qualitative and quantitative differences.5,6 Cells exposed to positive shear stress undergo reorientation, with their longitudinal axis parallel to the direction of blood flow.6,7 This reorientation streamlines the endothelial cell, decreasing the effective resistance and lowering shear stress,10 a phenomenon which may or may not be important in terms of adaptation or filtering of shear stress stimuli.7 At areas of abrupt curvatures...
in the vasculature, as in the carotid bifurcation, the laminar flow of blood is disrupted and separated flow patterns result. Specifically, the medial wall of the carotid bulb experiences higher shear stress, while the lateral wall experiences recirculation vortexes that vary with the cardiac cycle, resulting in flow reversal. Thus, the lateral area of the carotid bulb experiences oscillatory shear stress (periodic flow reversal with time-averaged shear stress approaching zero) and low mean shear stress. As a result of the low magnitude of the time-averaged shear stress, these cells do not reorient and may be exposed to high shear gradients (differences in shear stress magnitude on a cell scale) because their lack of streamlining yields a membrane topology in opposition to the mean shear vector. Several investigators have demonstrated that endothelial cells may actually be sensitive to the magnitude of the shear gradient. Whether these shear gradients or the time-averaged mean shear stress is more critical in terms of atherogenesis remains to be defined. Nevertheless, the significance of these flow patterns is demonstrated by studies that correlate development of atherosclerotic lesions (fatty streaks and small plaques) with areas of the carotid that experience these flow reversals with low time-averaged shear stress. Regions of the carotid bifurcation that experience pulsatile and mean positive shear stress as the result of laminar blood flow patterns, however, were relatively protected from atherosclerosis. Other investigators have confirmed these observations throughout the vasculature. The mechanism by which the physical force generated by fluid shear stress is transduced into biological signals remains unclear.

Below we will briefly review the atheroprotective effects of the endothelium that are influenced by shear stress and then discuss several signal-transduction mechanisms by which shear stress exerts its beneficial effects on endothelial function.

**Shear Stress and Endothelial Cell Biology: Relevance to Atherosclerosis**

The hypothesis that physical injury to the endothelium might precipitate the atherosclerotic process was introduced over two decades ago. More recently this concept has been modified to include biochemical and cellular alterations in endothelial cell function. There is a strong correlation between endothelial cell dysfunction and areas of low mean shear stress and oscillatory flow with flow reversal (Fig 1). Manifestations of dysfunctional endothelium can be readily observed in certain areas of the arterial tree, such as branch points, which experience low mean shear stress and flow reversal. These sites demonstrate increased uptake of lipoproteins, appearance of leukocyte adhesion molecules on the surface of the endothelial cells, and leukocyte transmigration. Secretion of chemotactic factors and growth factors causes proliferation of resident monocyte/macrophages, as well as smooth muscle cells. Smooth muscle cells synthesize a connective tissue matrix comprised of elastic fibers, proteins, collagen, and proteoglycans, and the accumulation of lipids and free and esterified cholesterol follows. Recent data suggest that low shear stress and, more importantly,
oscillatory flow and flow reversal are permissive or even causative in this pathogenic process.4,19

In areas downstream of vessel bifurcations, laminar shear stress predominates, and the endothelial cells experience pulsatile flow, with shear stress on the order of 10 to 30 dyne/cm².5 The endothelium in these regions maintains circulatory and blood vessel integrity through its ability to regulate several different processes: coagulation, growth of underlying smooth muscle, leukocyte adhesion to and transmigration into the blood vessel wall, and lipoprotein uptake and metabolism.

Coagulation
Coagulation stimulates the release of powerful antithrombotic agents from endothelial cells. Prostacyclin was the first inhibitor of platelet aggregation known to be released from endothelial cells on exposure to shear stress.7,14 Secretion of prostacyclin from endothelial cells is enhanced when the shear stress is pulsatile compared with steady.7 Numerous investigators have demonstrated that shear stress is one of the most powerful stimuli for release of the vasodilator NO,2,20,21 which also possesses strong anti–platelet aggregation properties.2,22 Shear stress can also stimulate release of factors that inactivate the clotting cascade.5 Recent studies have shown that shear stress regulates generation of thrombomodulin, which interacts with protein C and protein S to inactivate certain clotting factors. Malek et al.23 reported that steady shear resulted in a small transient increase in thrombomodulin expression but continued exposure to shear resulted in decreased thrombomodulin expression in bovine aortic endothelial cells. However, two other laboratories reported that steady shear stress results in sustained increased thrombomodulin expression in human umbilical vein endothelial cells.24,25 The reason for this disparity in species response is unclear. In addition to the potential effect on thrombomodulin, fluid shear stress has also been shown to stimulate expression of tissue plasminogen activator23,24,26 and reduce secretion of plasminogen activator inhibitor type-1.24 Importantly, endothelial cells exposed to turbulent flow failed to show increases in thrombomodulin and tissue plasminogen activator.

Leukocyte Adhesion and Migration
Endothelial cells regulate leukocyte adhesion and migration of monocytes and leukocytes into the blood vessel wall by secretion of chemotactic factors and expression of cell-surface molecules. ICAM-1 binds β2-integrins on various white blood cell derivatives, while VCAM-1 mediates adhesion of monocytes to the endothelium.27 VCAM-1 is one of the earliest markers for fatty streaks and is upregulated in areas of the endothelium surrounding atherosclerotic plaques.27 Several investigators have demonstrated an inverse relationship between VCAM-1 expression and shear stress,28–33 which suggests that leukocyte binding should be decreased under conditions of high shear stress. However, other investigators have demonstrated that ICAM-1 expression is upregulated by high shear stress34–36 and that leukocyte binding after exposure to shear stress is increased.35,36

The leukocyte binding experiments described above used static cultures of endothelial cells that were exposed to shear stress prior to the binding of leukocytes. However, these experimental conditions do not accurately simulate the physiological conditions of leukocyte binding and the complex interplay between expression of ICAM-1, VCAM-1, and the physical disruption of the leukocyte-endothelial cell interaction by high levels of shear stress.37,38 A study performed by Walpole et al.39 is helpful in analyzing which of these parameters may be of physiological importance. In this study, shear stress in rabbit carotid arteries was altered and expression of endothelial cell adhesion molecules, as well as leukocyte binding, was measured. Low shear stress resulted in VCAM-1 expression 30 times greater than that of control vessels, while ICAM-1 expression fell to approximately 30% of control vessels. High levels of shear stress also increased VCAM-1 expression (to 3.5 times that of control vessels), while ICAM-1 expression levels increased (to 1.6 times that of control). Importantly, extensive monocyte adhesion was noted under low shear stress, which colocalized to areas of VCAM-1 expression, while no monocyte adhesion was noted at high shear conditions, indicating that low mean shear stress promotes leukocyte binding in vivo compared with higher shear stress.

Another key factor in monocyte recruitment is the chemotactic peptide MCP-1.40 Shy et al.40 showed that MCP-1 expression was transiently increased in human umbilical vein endothelial cells on exposure to shear stress. However, MCP-1 gene expression then decreased to basal levels at 4 hours, and once gene expression was fully suppressed, it remained so even after static incubation, leading the authors to suggest that MCP-1 expression is likely suppressed in endothelial cells exposed to steady pulsatile shear stress. Thus, it seems probable that for conduit vessels, high shear stress inhibits leukocyte binding and chemotactic protein expression while low shear stress and flow reversal promote leukocyte binding and transmigration.

Proliferation
Smooth muscle proliferation is increased in atherosclerotic lesions4 and is likely stimulated by endothelial cell factors that are regulated by shear stress. Kraiss et al.41 showed that endothelial platelet-derived growth factor-A mRNA levels and smooth muscle proliferation were increased in areas that experience low blood flow in an arteriovenous fistula model of altered flow in baboons. Endothelin-1, a smooth muscle mitogen that acts synergistically with platelet-derived growth factor, was shown to be dramatically reduced by exposure to 25 dyne/cm².42 NO42 and transforming growth factor-β,43 both inhibitors of vascular smooth muscle cell growth, are secreted by endothelial cells in response to shear stress. Angiotsensin II is an important growth factor for vascular smooth muscle and may also be antiapoptotic.44 Shear stress regulates tissue levels of angiotensin II by virtue of changes in angiotensin-converting enzyme expression. Rieder et al.40 recently demonstrated that prolonged exposure to shear stress significantly reduced angiotensin-converting enzyme mRNA and activity. With its ability to regulate these disparate smooth muscle cell growth factors, it seems likely that shear stress plays a role in
the increased proliferation of smooth muscle seen at areas of low shear stress and flow reversal.

**Lipoproteins**

Unlike the effects of shear stress on growth factor secretion, the role of shear stress in lipoprotein transport and LDL metabolism is less well defined. Deng et al reported that the concentration of LDL at the surface of canine carotid arteries was inversely related to wall shear stress rate and suggested that increased surface LDL concentration results in an increased rate of lipid infiltration into the blood vessel. This hypothesis is complemented by studies which demonstrate that areas exposed to flow reversal are relatively permeable to macromolecules including LDL and that LDL accumulation within the vascular wall is preferentially localized to these areas of disturbed flow. Berceli et al reported that the LDL incorporation in the rabbit aorta–iliac bifurcation was elevated in the lateral region that experiences flow reversal versus the medial regions that experience higher steady shear, while no differences were present in the transitional or unidirectional zone that experiences relatively steady shear. Other investigators have confirmed these findings in different areas of the vasculature for both rabbits and pigs. Mechanistically, it appears that compromised endothelial cell integrity, and hence increased macromolecule permeability, results from high shear gradients that are present in low shear stress/flow reversal conditions (See Weinbaum and Chien for review). A study by Sprague et al showed that 125I-LDL internalization increased in bovine aortic endothelial cells exposed to steady stress conditions for 24 hours; however, this likely reflects an increased metabolic need for LDL under steady shear conditions rather than increased LDL incorporation into the arterial wall. Additional studies to define the mechanistic details by which LDL accumulation is linked to low shear stress and flow reversal conditions are warranted.

**Endothelial Cell Survival**

Finally, shear stress may be critical for endothelial cell survival. Early studies performed by Davies et al demonstrated increased endothelial cell turnover in areas that experience turbulent shear stress conditions, suggesting compromised endothelial cell integrity under these conditions. Several recent studies report that the lack of shear stress triggers apoptosis in endothelial cells. Other investigators have demonstrated that shear stress is required for optimal regeneration of an injured endothelium. Vyalov et al reported that under low shear stress conditions, endothelial cells on the border of a wound edge failed to maintain contact with neighboring cells and were oriented randomly. Further, the cells spread and migrated into wound sites more slowly. While steady shear seems to be necessary for endothelial cell integrity, several investigators have demonstrated that steady shear inhibits proliferation of cultured endothelial cells. Thus, it appears that shear stress acts as an endothelial cell “survival” factor rather than as a “growth” factor.

**NO: A Critical Factor in Shear Stress–Mediated Atheroprotection**

NO appears to be a key mediator of the atheroprotective effects of shear stress on the blood vessel wall. NO has been reported to play a role in platelet aggregation and leukocyte binding to the endothelium, in inhibition of vascular smooth muscle tone and growth, and in alteration of lipoprotein metabolism. The ability of shear stress to regulate these processes is abrogated by inhibitors of NO production, suggesting that shear stress exerts its effects through the release of NO. Further, it has been postulated that the beneficial effects of regular aerobic training, including its antiatherogenic properties, may be mediated through shear-induced increases in NO secretion.

NO is produced by a unique enzyme present in the endothelium, termed ecNOS. Shear stress is the most potent physiological stimulus for NO production in endothelial cells. Rapid increases in NO production are due to posttranslational activation of ecNOS, while chronic alterations in ecNOS expression are due to changes in gene expression. Experiments by our laboratory and others indicate that two distinct signaling pathways (a Ca2+-dependent and a Ca2+-independent pathway) seem to be involved in rapid shear-mediated increases in NO production. We compared NO production in response to the Ca2+ ionophore A23187 with shear stress. While A23187 increased NO production by 3-fold to 6-fold, shear stress stimulated NO production by 10-fold to 30-fold above static levels. The initial rapid increase in NO required Ca2+, while the sustained increase in NO production was independent of changes in intracellular Ca2+. Further experiments by our laboratory have demonstrated that ecNOS was phosphorylated in response to shear. Although the relationship between ecNOS phosphorylation and NO production is unclear, phosphorylation may regulate the activity of ecNOS. To better understand how shear stress influences ecNOS activity and expression, it will be necessary to identify upstream mediators of ecNOS function that are activated by shear stress, such as protein kinases.

**Mitogen-Activated Protein Kinases: Likely Signaling Molecules in the Transduction of Shear Stress**

Several features of the endothelial cell response to shear stress are analogous to receptor-mediated signaling: dependence on G proteins, increase in intracellular Ca2+, and changes in gene expression. The family of kinases termed MAP kinases are potential candidates to mediate some of the effects of shear stress on endothelial cells. MAP kinases are ubiquitously expressed serine/threonine protein kinases that are activated in response to a variety of extracellular stimuli involved in cell growth, transformation, and differentiation (Fig 2). The extracellular signal–regulated kinases (ERK1/2), members of the MAP kinase family, have many potential substrates, including other protein kinases (p90rsk, MAPKAP, Raf-1, MEK), transcription factors (c-myc, c-jun, c-fos, p62TCF), enzymes (cPLA2), and cell-surface proteins (EGF receptor), and thus have many effects on cellular physiology and gene expression.

The pathway for ERK1/2 activation in response to growth factors has been well characterized (Fig 2). The MAP and ERK kinase (MEK-1) is a dual-specificity kinase that phosphorylates ERK1/2 on T-E-Y. MEK-1 is itself regulated by a MAP kinase kinase kinase, one of which has been identified...
as Raf-1. Raf-1 is activated by translocation to the membrane and association with the small GTP-binding protein, ras. The GTPase activity of ras is regulated by a complex involving Grb2 and mSOS which are recruited and activated by a tyrosine kinase receptor.65

We have recently reported that ERK1/2 is activated by shear stress in endothelial cells in a time- and force-dependent manner.66 Shear stress stimulation of ERK1/2 was unaffacted by treatment with the Ca\(^{2+}\) chelator BAPTA-AM, indicating the response was Ca\(^{2+}\) independent. These data, combined with observations that ecNOS contains multiple consensus sites for phosphorylation by a variety of kinases including ERK1/2,63 make this pathway a likely candidate to participate in the stimulation of sustained NO production in response to shear stress. Additionally, several shear stress-responsive genes contain elements (eg, AP-1)39,67 that may be influenced by ERK1/2-mediated phosphorylation of transcription factors,63 such as c-fos, c-jun, and c-myc.

Another member of the MAP kinase family shown to be regulated by shear stress is the stress-activated protein kinase JNK/SAPK. Two laboratories have shown increases in JNK activity by shear stress, although with varying kinetics.68,69 Preliminary results in our laboratory show that activation of PKC isoforms, particularly PKC-\(\varepsilon\), is Ca\(^{2+}\) independent. Thus, it seems likely that PKC-\(\varepsilon\) is the "classical" PKC isoform necessary for the ERK1/2 stimulation by shear stress.66

Figure 2. MAP kinase activation pathways. A common theme in the stimulation of MAP kinase family members is activation by an immediate upstream MAP kinase kinase (MEK), which is in turn activated by an immediate upstream MAP kinase kinase (MEKK). Different stimuli activate different signaling pathways, leading to individual MAP kinase activation.

Figure 3. Proposed model of shear stress-mediated mechanotransduction in endothelial cells. Primary mechanosensors (eg, integrins, caveolae, G proteins, ion channels) transduce physical stimuli into biochemical signals. Several stimuli serve to activate Raf-1, including tyrosine phosphorylation by c-Src or c-Src-like kinases, serine and threonine phosphorylation by PKC, and GTP-bound ras. Raf-1 activates MEK, which in turn activates ERK1/2. Sustained generation of NO may result from the effects of ERK1/2 or through direct effects of mechanosensors (eg, caveolae) themselves.

Upstream Effectors of ERK1/2 Activity
PKC is required for ERK1/2 activation in response to shear stress because the ERK1/2 activation by shear stress reported by our group was attenuated when endothelial cells were pretreated with either phorbol ester for 24 hours or with staurosporine for 30 minutes66 (Fig 3). PKCs are well characterized serine/threonine kinases that are activated by a variety of stimuli.70 A classification system for the PKC family separates the different isoforms into distinct classes: the "classical" PKC isoforms, (PKC-\(\alpha\), -\(\beta\)I, -\(\beta\)II, -\(\gamma\)) are Ca\(^{2+}\) independent and phorbol ester responsive; the "novel" PKC isoforms (PKC-\(\delta\), -\(\epsilon\), -\(\theta\), -\(\eta\)) are Ca\(^{2+}\) independent and phorbol ester responsive; and the "atypical" PKC isoforms (including \(\zeta\), \(\lambda\), \(\nu\), \(\tau\)), are Ca\(^{2+}\) independent and phorbol ester unresponsive. Human umbilical vein and bovine aortic endothelial cells express primarily three PKC isoforms: PKC-\(\alpha\), PKC-\(\epsilon\), and PKC-\(\zeta\).71 Through experiments using antisense oligonucleotides to inhibit expression of the various PKC isoforms in endothelial cells, we identified PKC-\(\epsilon\) as the isoform necessary for the ERK1/2 stimulation by shear stress.71

A recent study by Cai et al72 provides evidence for the mechanism by which PKC activates ERK1/2. These investigators demonstrated that activation of the MAP kinase kinase kinase, Raf-1, a key activator of ERK1/2, is dependent on several criteria: (1) recruitment of Raf-1 to the plasma membrane; (2) activation of Raf-1 by the GTP-bound form of ras; (3) tyrosine phosphorylation of Raf-1, presumably by c-Src or a c-Src-family kinase; and (4) phosphorylation of Raf-1 on serine and threonine residues. Both PKC-\(\alpha\) and PKC-\(\epsilon\) were directly responsible for phosphorylation of serine and threonine residues on Raf-1 in response to various stimuli. This redundancy in PKC signaling to Raf-1 would allow for signaling regardless of a rise in intracellular Ca\(^{2+}\), as PKC-\(\epsilon\) is Ca\(^{2+}\) independent. Thus, it seems likely that activation of PKC isoforms, particularly PKC-\(\epsilon\), by shear stress results in stimulation of ERK1/2 through their action on Raf-1, regardless of intracellular Ca\(^{2+}\) concentration.
Potential Shear Stress Receptors

A question of great importance in the field of mechanotransduction pertains to the identity of the primary mechanoreceptor(s) responsible for initiating signal transduction. Transduction of mechanical forces in anchorage-dependent cells is due to a combination of force transmission via the cytoskeletal elements and transduction of the physical forces to biochemical signals at mechanotransducer sites. Based on the data presented above, the candidate mechanotransducer molecules should be responsive to shear stress over the physiological range and result in the activation of a tyrosine kinase (eg, c-Src), PKC, and ERK1/2. Due to their interaction with specific signaling molecules already implicated in signal transduction, four candidates have been proposed as likely mechanotransducers: integrin-matrix interactions, specialized membrane microdomains, ion channels, and G proteins.

To sense and transduce signals in response to shear stress, endothelial cells must be anchored to their matrix. Integrins are ubiquitous α/β heterodimeric transmembrane glycoproteins that act as adhesion receptors involved in the interaction between cells and extracellular matrix. Integrins play an important role in biological processes, including cell adhesion, cell migration, cell growth, tissue organization, blood clotting, inflammation, target recognition by leukocytes, and cell differentiation. Studies performed by Wang et al and Ingber using magnetic torsion have demonstrated that integrins are capable of transducing mechanical stimuli to biochemical signals. A recent study by Muller et al showed that flow-induced vasodilation in coronary arteries, which is mediated by NO release, could be blocked with RGD peptides, which compete with the matrix for integrin interactions. Similar attenuation of flow-induced vasodilation was obtained if a blocking antibody against the β3 integrin was employed, supporting the hypothesis that integrins are involved in the mechanotransduction of shear stress. Integrins are also a particularly attractive candidate in that they have been reported to associate with PKC and c-Src–family tyrosine kinases. Through the use of antisense oligonucleotides against the various PKC isoforms, our laboratory has shown that adhesion-mediated activation of ERK1/2 in human umbilical vein endothelial cells is dependent on PKC-α and PKC-ε, a finding that parallels the data from Cai’s group. Other studies by our laboratory have demonstrated that activation of β1 integrins (the predominant β isoform on endothelial cells) with an activating antibody also stimulated ERK1/2, although at levels less than that observed with shear stress. Further, human endothelial cells showed adhesion-mediated ERK1/2 activation when plated on a matrix of fibronectin, which engages β1 integrins, but showed no ERK1/2 activation when they adhered to matrix consisting of poly-L-lysine. The relatively small magnitude of ERK1/2 stimulation by integrin activation does not preclude a key role for integrins in shear-mediated ERK1/2 activation; based on the importance of shear stress to endothelial cell function and integrity, it is likely that redundant pathways with different mechanotransducer molecules mediate the full ERK1/2 response to shear stress.

Another possible candidate for the transduction of shear stress into biochemical signals are caveolae, specialized domains of the plasma membrane that are rich in cholesterol. Because of their high cholesterol content, caveolae are more rigid than other portions of the plasma membrane. Caveolae are abundant in endothelial cells and have been implicated in transcytosis, ion movement across the membrane, and signal transduction. The principal component of caveolae is a 21- to 24-kD integral membrane protein called caveolin. Caveolin seems to function as a scaffold for the recruitment and sequestration of signaling molecules. Among signaling molecules known to associate with caveolae are G proteins, c-Src–family tyrosine kinases, ras, PKC, ecNOS, shc, Grb2, mSOS, Raf-1, and ERK1/2 (see Reference 82) Caveolae represent an attractive site for mechanotransduction on the basis of their biophysical characteristics and interactions with signaling molecules. Experiments to determine the significance of caveolae and what effect changes in caveolae number and properties may have in shear-mediated signaling should prove an exciting area for future research.

Recent data reported by Gudi et al indicate that G proteins may act as primary mechanosensors in endothelial cells. This laboratory showed that treatment of endothelial cells with antisense Goq oligonucleotides inhibited shear stress–induced ras-GTPase activity, while scrambled oligonucleotide treatment had no effect. Another study reported that treatment of endothelial cells with pertussis toxin prevented shear stress–mediated activation of ERK1/2, also suggesting that G proteins are activated in response to shear stress. Further, Gudi et al demonstrated that G proteins reconstituted in liposomes, in the absence of protein receptors, showed an increase in activity in response to shear stress. This shear stress–mediated increase in G protein activity could be attenuated if the lipid bilayer was made more rigid by the addition of cholesterol, a significant finding in the context of caveolae as shear stress signaling domains.

A common mechanism that has evolved to sense changes in mechanical stimuli are the mechanosensitive ion channels. These channels are widely distributed in tissues and participate in processes such as hearing, balance, and reflex contraction of both smooth and skeletal muscle. Endothelial cells exhibit ion channel responses to mechanical forces that are likely to participate in the signaling response to shear stress. Several different mechanosensitive ion channels are present in endothelial cells, including a shear-responsive K+ channel and a stretch-activated Ca2+ channel. Studies have shown that blockade of mechanosensitive K+ channels with barium chloride or tetraethylammonium blocked shear-mediated increases in NO production and transforming growth factor-β release, suggesting that transmembrane ion flux and intracellular ion homeostasis are important mediators of the endothelial cell response to shear stress. However, efforts to clone the mechanosensitive K+ channel from the endothelial cell have not yet been successful.

Based on the demonstrated importance of shear stress to endothelial cell function and integrity, it is likely that each of these putative mechanoreceptors activates intracellular signaling pathways to effect the complete endothelial response to shear stress. Differential coupling of signaling mechanisms and subsequent endothelial cell response to the individual shear stress receptor “subtypes” may provide a flexibility to
the endothelial cells in terms of responding to varying types and degrees of shear stress that they may encounter.

Conclusions
We have reviewed data showing that shear stress has direct influences on the pathogenesis of atherosclerosis via regulation of endothelial cell function and integrity. Shear stress influences many of the processes relevant to development of the atherosclerotic lesion, including secretion of growth factors, regulation of coagulation, and transmigration of leukocytes. Regulation of these processes (Fig 3) is proposed to occur via shear-activated endothelial cell signal-transduction pathways that involve primary mechanotransducers, resulting in the activation of ERK1/2, and possibly eNOS, through signaling molecules such as nonreceptor tyrosine kinases, ras, and PKC.

While hemodynamic considerations are important in atherogenesis, it is unlikely that fluid mechanical forces are the sole positive or negative atherogenic stimuli. The potential influence of local and systemic biochemical factors and their interplay with mechanical factors must also be considered.65,67 Apart from the direct effects of shear stress on endothelial cell function, flow reversal results in alterations in mass transport, increasing the probability of leukocyte localization and altering delivery of biochemical factors such as inflammatory mediators that may contribute to the local atherogenic state. The conclusion that the hemodynamic force of fluid shear stress plays an important role in the pathogenesis of atherosclerosis, however, does provide a framework by which independent risk factors for atherosclerosis may be understood. Studies suggest that hypertension,88 diabetes,89 and hypercholesterolemia68 promote atherosclerosis by disrupting the ability of the endothelium to respond to shear stress, while regular aerobic exercise exerts atheroprotective effects through shear-mediated increases in NO.57 Further elucidation of the mechanisms of shear stress-mediated signal transduction and its alteration with these risk factors will greatly advance our understanding of atherosclerosis.

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