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MOLECULAR AND BIOLOGICAL EFFECTS OF HEMODYNAMICS ON VASCULAR CELLS

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1. ABSTRACT

A variety of systemic risk factors, including smoking, hypertension, hyperlipidemia and diabetes have been found to promote atherosclerosis. Although these elements affect blood vessels equally, clinically significant lesions develop at predictable locations, i.e., major branch points and bifurcations. This suggests that the development of clinically significant atherosclerotic plaques involves a complex interplay between vascular anatomy, vascular biology and hemodynamic forces. Cyclic strain, circumferential pulsatile pressure exerted upon a vessel wall, has been found to cause changes in endothelial cells that tend to disfavor atherosclerosis formation. Cultured endothelial cells have been shown to migrate, proliferate and alter cytoskeletal alignment in response to cyclic strain. Levels of macromolecules such as prostacyclin, endothelin, nitric oxide and tissue plasminogen activator have been found to be altered by cyclic strain. Additionally, cyclic strain has been shown to stimulate expression of cellular adhesion molecules such as ICAM-1 and intracellular second messenger systems such as the adenylate cyclase-cAMP, diacylglycerol-IP_3, and protein kinase C pathways. This article reviews the most current pertinent literature and summarizes the presently known effects of cyclic strain on endothelial cells.

2. INTRODUCTION

Atherosclerosis is a chronic disease with systemic risk factors. Smoking, hypertension, hypercholesterolemia, and diabetes are processes that affect the vasculature as a whole. Atherosclerotic plaques are characterized by the accumulation of cholesterol, macrophages, smooth muscle cells (SMC), extracellular matrix (ECM) proteins and thrombus in the intimal layer of the vessel wall. As the plaque increases in size, the lumen of the vessel narrows until it is completely occluded by plaque and thrombus. The formation of atherosclerotic plaque involves multiple interrelated mechanisms. The initiating event is the activation of endothelial cells, which has several consequences (Figure 1). Exposure of subendothelial collagen facilitates platelet attachment, aggregation, and EC secretion of a variety of cytokines, including platelet derived growth factor (PDGF). PDGF acts directly on vascular SMC to cause them to proliferate and migrate to the intima, which is a key event of atherosclerosis. (1) Intimal SMC produce transforming growth factor-beta (TGF-β), which acts in an autocrine fashion to cause the secretion of collagen and other proteins into the extra-cellular matrix (ECM). (2) Concurrent damage to the endothelium impairs its barrier function, allowing for deposition of cholesterol and migration of macrophages and lymphocytes into the intima. Further endothelial damage by the growing plaque impairs the secretion of nitric oxide (NO), prostacyclin (PGI2) and tissue plasminogen activator (tPA) which results in the propagation of thrombus. (3)

3. LOCALIZATION OF ATHEROSCLEROSIS

The lesions of atherosclerosis, interestingly, localize in distinct sites within the vasculature, especially affecting the coronary arteries, the major branches of the aortic arch, and the abdominal aorta and its visceral and major lower extremity branches (Figure 2). (4) The carotid bifurcation is a good example of this localizing process. Plaque formation occurs at the origin of the internal carotid artery, whereas, the distal internal carotid artery and the proximal common carotid artery do not demonstrate any plaque formation. (5) The configuration and branch angle of the internal carotid sinus produce an area of altered hemodynamics along the outer wall of the vessel, where atherosclerotic plaques tend to form. (6) In one study, human carotid bifurcations obtained at autopsy were used to construct glass and plexiglass models for flow visualization and velocity measurements with laser doppler anemometry. Using quantitative measurements, the authors were able to correlate the presence (or absence) of atherosclerosis in the cadaveric carotid bifurcation

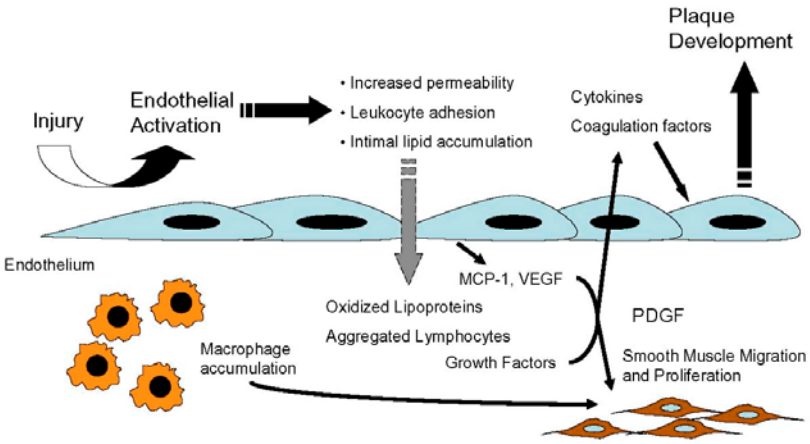

Figure 1. The Process of Atherosclerosis. Endothelial cell activation leads to a cascade of events that leads to the eventual formation of an atherosclerotic plaque.

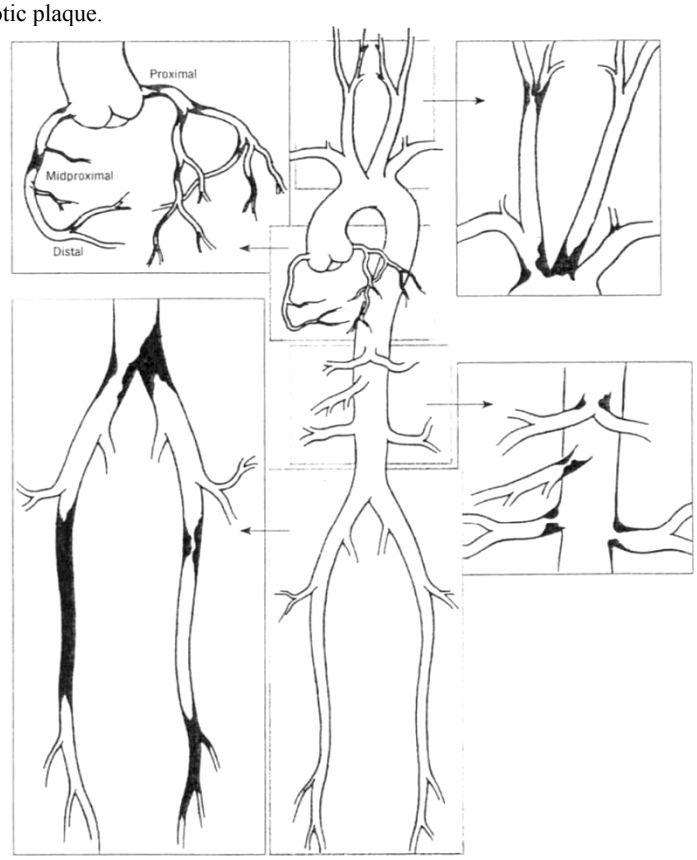

Figure 2. Localization of Atherosclerotic Plaques. Atherosclerotic plaques tend to form at branch points and vessel bifurcations.

specimens with fluid velocity and flow patterns observed in the constructed models. Their findings showed that areas of low wall shear stress, flow separation and flow oscillation were associated with atherosclerotic plaque formation. Further analysis demonstrated that plaque formation was always most significant in the proximal and mid-sinus sections of the internal carotid artery. (7)

Studies of human hearts have shown that the proximal sections of the left anterior descending (LAD) and left circumflex coronary arteries (LCx) are, similarly, prone to forming atherosclerotic plaques at branch points. (4) Since these vessels are just distal to the bifurcation of the left main coronary artery (LM), it is thought that local hemodynamic forces render them susceptible to plaque formation. In one study, model casts were constructed from cadaveric human coronary artery specimens, and were analyzed with a computer-based algorithm. Comparisons were made between the branch angle of the LAD and LCx junction and the presence of atherosclerosis. Results of this comparison showed that the formation of plaque was greater in vessels where the branch angle was larger. (8)

Bifurcations with larger branch angles have higher velocity gradients between their inner and outer segments which results in a greater intensity of flow disturbances. Consequently, there is a greater tendency for atherosclerotic plaque formation in those vessels. Additional research, using a computer generated model to analyze coronary angiograms of the LCA, showed that flow velocity profiles were skewed to the inner wall of the branching vessel, causing a high shear laminar flow pattern there. At the lateral walls of the bifurcation, there was a reduction in shear stress, accompanied by abrupt changes in flow velocity and oscillations in flow direction, conditions favoring the formation of atherosclerotic plaques. (9) The coronary vessels are more likely to demonstrate oscillatory flow patterns and other hemodynamic disturbances which may render them susceptible to atherosclerosis. (5)

Clinically significant atherosclerosis is common in the abdominal aorta, but is less frequently observed in the thoracic aorta. In the abdominal aorta, atherosclerosis is predominantly located along the posterior wall of the aortic bifurcation, and extends proximally to the renal arteries. There is commonly a short infrarenal segment of aorta which is free of disease. (10) The presence of atherosclerosis in the abdominal aorta is thought to be due to the different hemodynamic conditions which exist in the thoracic and abdominal aorta. In one study using an anatomically accurate model, flow velocity profiles were analyzed for different sections of the aorta. In the thoracic aorta, there is a strong biphasic forward flow pattern with minimal retrograde flow. In contrast, the flow patterns observed in the abdominal aorta distal to the renal arteries demonstrate significant regions of flow disturbance and low shear stress. Maximal flow velocities are observed at the anterior wall, with significant flow reversal occurring at the posterior wall, particularly in diastole. (11) The presence of retrograde flow and oscillatory flow patterns in the infrarenal aorta coincide with the characteristic localization of atherosclerotic plaques in that vessel.

Further investigation using *in vivo* models has shown a relationship between areas of low wall shear stress and formation of atherosclerotic lesions. Studies using human autopsy specimens have compared intimal thickness at multiple locations in the abdominal aorta to flow velocity patterns measured by MR imaging. The results showed that low and oscillating wall shear stresses were associated with regions of intimal thickening and the development of atherosclerosis. (12) In another study, a hyperlipidemic swine model was used. A 50% aortic stenosis was created in the animals which were then fed a hyperlipidemic diet. Doppler ultrasound was used to measure blood flow velocities at specific locations in the modified aorta. Local wall shear stress was calculated using flow velocity profiles and linear regression analysis. The aortas were procured after seven months and plaque thickness was compared between the stenosed and non-stenosed side and correlated with shear stress values. The stenosed side exhibited regions of disturbed flow and low wall shear stress, with increased formation of atherosclerotic plaques. In contrast, the non-stenosed side demonstrated laminar flow patterns and high wall shear stress, with significantly less plaque formation on that side. These novel studies, with each animal serving as its own control, support the hypothesis that low wall shear stress and altered hemodynamics facilitate atherosclerotic plaque formation. (13)

Earlier thinking in vascular biology implicated high shear stress as a causative factor in atherosclerosis. This was based on the hypothesis that high shear stress caused the endothelial injury and SMC proliferation which accompanied atherosclerosis. (14) However, subsequent research has not supported this premise. Current belief is that atherosclerotic plaques form in regions of low shear stress and disturbed flow. Using both *in vivo* and *in vitro* models, it has been demonstrated that vessels which are prone to plaque formation have non-laminar flow patterns characterized by low shear stress, flow separation and stasis, and oscillations of flow. Persistent elevations of shear stress are not associated with endothelial injury, and regions of high shear stress tend not to form atherosclerotic plaques. Intimal thickening and atherosclerosis are observed in vessel branch points and bends, which produce local alterations of hemodynamic conditions.

Analogous studies focusing on mechanical stress have shown that regions which are susceptible to plaque formation demonstrate alterations in pressure-induced wall tension, that is, cyclic strain. One group, using an *in vivo* canine iliac artery bifurcation model, demonstrated that wall tension measured at branch origins is 4 to 6 times greater than in other regions. This creates focal areas of mechanical stress concentration at both the proximal and distal edge of the vessel ostium. Elevation of wall tension and mechanical stress concentrations in these region leads to increased cyclic strain of EC and SMC. (15)

An animal study using a rabbit model investigated the effect of cyclic strain on endothelial cell morphology at arterial branch sites. Examination of EC from straight sections of the aorta show a smooth layer of intact EC with normal morphology and cell orientation. In contrast, EC at branch sites have abnormal spindle and cobblestone shapes and disordered orientation. (16) The endothelial lining has an important function in preventing plaque formation by acting as a barrier against the deposition of cholesterol and inflammatory cells in the intima. Disruption of EC orientation and alignment is likely to be an important step in the pathogenesis of atherosclerosis. The implication of the above evidence is that repetitive cyclic strain of the vessel wall is a causative factor for atherosclerosis. But this is likely an oversimplification of the role of cyclic strain in vascular cell biology.

4. HEMODYNAMIC FORCES

Arterial blood vessels are subjected to major hemodynamic forces which impact the endothelial cell lining. The endothelial cell monolayer is an active participant in the complex interactions that occur between the luminal blood and vessel wall. As previously eluded to, it is the biologic response of the endothelium to hemodynamic forces that is pivotal in the process of

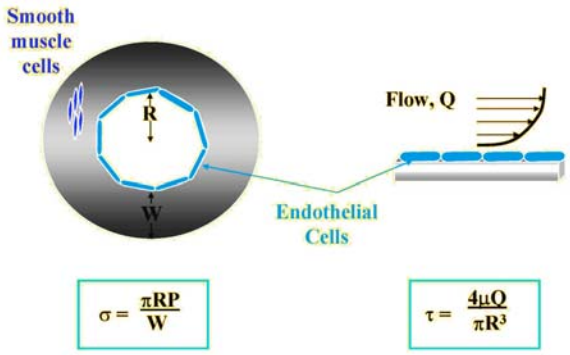

Figure 3. Hemodynamic Forces. Endothelial cells are subjected to both a tangential, parallel force (shear stress) as well as a circumferential, perpendicular force (cyclic strain).

therosclerosis. The arterial blood vessel is subjected primarily to two major hemodynamic forces: shear stress and cyclic strain (Figure 3). As blood moves along the endothelium, a tangential drag force is produced called shear stress. (17, 18) The magnitude of the shear stress is directly proportional to blood viscosity and inversely proportional to the radius of the blood vessel cubed. Research has shown that high shear stress is inversely proportional to the distribution of early intimal lesions. Results of these studies demonstrate that endothelial cells respond to shear stress on a macromolecular basis as well as on an intracellular basis (Table 1). Shear stress has been shown to cause endothelial cells re-alignment in the direction of flow via reorganization of endothelial cell F-actin and inhibition of endothelial cell migration and proliferation. (17) In addition, shear stress affects the biologic function of endothelial cells and results in increased prostacyclin,(19, 20) tissue plasminogen activator, and nitric oxide levels. (21, 22)

Cyclic strain refers to the repetitive, circumferential pulsatile pressure distention conferred to the vessel wall. As with shear stress, cyclic strain causes certain functional and structural responses when applied to vascular endothelial cells (Table 2). Cultured endothelial cells have been shown to migrate, proliferate and exhibit morphologic changes in response to cyclic strain. The morphologic changes occur secondary to actin rearrangement within the cytoskeleton resulting in an organized cellular alignment perpendicular to the force vector. (23, 17) Several macromolecules have been found to be stimulated by cyclic strain. As with cells that are subjected to shear stress, endothelial cells undergoing cyclic strain exhibit increased levels of prostacyclin, endothelin, and tPA. In addition, endothelial nitric oxide synthase and subsequently, nitric oxide levels are also increased. (24-26) Moreover, cyclic strain has been shown to stimulate expression of cellular adhesion molecules such as ICAM-1(27) and intracellular second messenger systems such as the adenylate cyclase-cAMP, diacylglycerol-IP_3, and PKC pathways (Table 3). (28)

The arterial EC monolayer transduces mechanical signals for the vessel wall; local hemodynamic forces stimulate vascular ECs and initiate pathways which affect the entire vessel. Recently, the impetus in the field has shifted from a description of phenotypic modifications of ECs exposed to external forces to a more mechanistic investigation of the biochemical pathways which link cell surface events to nuclear responses. (29) This review summarizes current research, which has aimed to elicit the signal transduction pathways by which cyclic strain elicits functional and structural responses in ECs.

5. EFFECTS ON THE EXTRACELLULAR MATRIX AND VASCULAR CELLS

Integrins are cell surface membrane proteins on vascular EC and smooth muscle cells (SMC) which anchor the cell to the extracellular matrix (ECM) and function as mechanotransducers, capable of coupling outside forces to biochemical signals. The binding of integrins to the ECM serves to transmit mechanical forces to the cell. ECM-integrin interactions mediate the cellular response to cyclic strain by activating MAP kinases. (30, 31) Studies have showed that cyclic strain affects DNA synthesis in cells cultured on certain ECM proteins but not on others. Specifically, cyclic strain increased DNA synthesis in cells grown on collagen, fibronectin, or vitronectin, but not on laminin or elastin. (32) These results suggest that the ECM has a functional role in mediating the activation of MAP kinases and the cellular proliferation response to mechanical forces.

Matrix metalloproteinases (MMPs) are hypothesized to be involved in the processes of endothelial cell (EC) migration and matrix remodeling during angiogenesis. The effects of hemodynamic forces on the regulation of MMPs including membrane type 1 matrix metalloproteinase (MT1-MMP) was studied in our lab. Rat microvascular EC were exposed to 60 cycles/minute of 24% maximum strain for up to 24 hours. MT1-MMP mRNA and protein increased in a time-dependent manner through 24 hours of exposure to cyclic strain. Cyclic strain induced early growth response gene product (Egr-1) mRNA and protein within 1 hour. A specific nucleoprotein complex was formed when an oligonucleotide containing binding sites for Sp1 and Egr-1 was incubated with nuclear extracts from EC exposed to 1 hour of cyclic strain. Antibodies to Egr-1 completely supershifted this complex. Increased binding of Egr-1 by cyclic strain to the MT1-MMP promoter correlated with enhanced transcriptional activity. These results suggest that cyclic strain up-regulates the Egr-1-mediated expression of MT1-MMP in rat microvascular EC, emphasizing the importance of hemodynamic forces in the regulation of MT1-MMP *in vivo*. (33)

Cyclic strain has been found to induce the reorganization of vascular EC integrins, specifically $\alpha_5\beta_1$ [a fibronectin receptor] and $\alpha_2\beta_1$ [a collagen-I receptor]. Yano *et al.* showed that β_1 integrin reorganized in a linear pattern with the long axis of the elongated cells, creating a fusion of focal adhesions in collagen- or fibronectin-plated ECs after 4 hour strain exposure. Cyclic strain also led to the reorganization of α_5 and α_2 integrins in a linear pattern in

Table 1. Effects of shear stress on endothelial cells

	RESPONSE TO SHEAR STRESS
PROLIFERATION	INCREASE; Turbulent flow
ALIGNMENT	PARALLEL
F-ACTIN REDISTRIBUTION	YES
FOCAL ADHESION SITE MODIFICATION (FAK)	YES
PROSTACYCLIN (PGI2)	INCREASED
NO	INCREASED
tPA	INCREASED
ENDOTHELIN-1	INCREASED/DECREASED
INTRACELLULAR CALCIUM	INCREASE--Activation of Ca^{++} sensitive pathways.
INOSITOL TRIPHOSPHATE (IP_3)	INCREASE--Phosphoinositide sensitive pathways.
CYCLIC GMP (cGMP)	INCREASE—Vasoregulation mechanisms.
MAP KINASE	INCREASE--Involvement of membrane mitogen receptor-like pathway

Table 2. Effect of cyclic strain on endothelial cells

	EFFECT OF CYCLIC STRAIN
PROLIFERATION	INCREASE
ALIGNMENT	PERPENDICULAR
F-ACTIN REDISTRIBUTION	YES
FOCAL ADHESION SITE MODIFICATION (FAK)	YES
INTEGRINS	REORGANIZE
PROSTACYCLIN (PGI2)	INCREASED
NO	INCREASED
tPA	INCREASED
ENDOTHELIN-1	DECREASED
PDGF	INCREASE
VEGF	INCREASE

Table 3. Effect of cyclic strain intracellularly on endothelial cells

	RESPONSE TO CYLIC STRAIN
Intracellular calcium	INCREASE—Activation of Ca^{++} sensitive pathways.
IP_3	INCREASE—Phosphoinositide sensitive pathways.
Diacylglycerol	INCREASE—Activation of the PKC pathway.
PKC	INCREASE—Phosphoinositide sensitive pathways.
Adenylyl cyclase	INCREASE—Activation of the cAMP pathway.
cAMP	INCREASE—Activation of the cAMP pathway.
PKA	INCREASE—Activation of the PKA pathway.
MAP Kinase (ERK/JNK/p38)	INCREASE—Involvement of membrane mitogen receptor-like pathways.
ICAM-1	INCREASE—Promotion of cellular adhesion.
ras	INCREASE—Involvement of extracellular mitogenic pathways.
Src	INCREASE—Transduction of biomechanical information.
Akt	INCREASE—Activation of pro-survival pathways.

ECs seeded on fibronectin or collagen, respectively; once again, integrins were more concentrated and aligned along the long axis of ECs creating a large fusion of focal adhesions. The expression of integrins α_5, α_2, and β_1, however, did not change even after 24 hour exposure to strain. This was the first report that strain reorganizes and fuses integrins in ECs without affecting their levels of surface expression. (34)

The tyrosine phosphorylation of cytoplasmic PTKs serves as a method for signal transmission into the cell. Integrins play an important role in transducing cyclic strain stimulation into intracellular signals; one potential mechanism, by which integrins affect signal transduction, is through their ability to initiate the process of phosphorylation on tyrosine residues of cytoplasmic kinases. A major substrate for integrin-induced tyrosine phosphorylation is the PTK focal adhesion kinase (FAK or $pp125^{FAK}$), whose autophosphorylation leads to the activation of other PTKs. (35) The clustering of β_1 integrin is known to induce FAK phosphorylation(36, 37) via an undefined mechanism. Yano *et al.* speculate that the integrin α subunit acts as a mechanotransducer by sensing and responding to strain, while the β_1 integrin cytoplasmic domain relays the signal into the cell. (34)

The effects of cyclic strain on EC secretion of vasoactive molecules are similar to those observed for shear stress. It is thought that physiologic levels of mechanical strain, manifested by the cyclic stretching of

vascular EC during systole, cause the secretion of NO, PGI2, and tPA. Basal levels of these substances, which have vasodilatory, antiplatelet and antithrombin effects, are thought to be necessary to inhibit the development of atherosclerotic plaques.

The synthesis of NO by EC is a product of arginine metabolism, and is catalyzed by the enzyme NO synthase. (38) In our laboratory, we have studied the effect of cyclic strain on NO synthase in cultured bovine EC. We have shown that cyclic strain causes an increase in both the production of NO synthase and in the functional activity of the enzyme. (39) At the nuclear level, cyclic strain causes an increase in the transcription of the eNOS gene. There is evidence to show that this activity is mediated by MAP kinases.

Studies have demonstrated that endothelial cells which are exposed to physiologic levels of cyclic strain increase their secretion of tPA. (40) As previously described, tPA has important functions in limiting the development of atherosclerosis. Basal levels of tPA are involved in the degradation of fibrin and thrombin, as well as collagen and other ECM proteins. The underlying signal transduction pathway for tPA secretion is not entirely clear, but is thought to involve protein kinase C and the inositol triphosphate/diacylglycerol (IP3/DAG) pathways. There is evidence to show that IP3 mediated calcium release is required for tPA secretion. Other studies have suggested that DAG and protein kinase C regulate tPA production at the level of transcription. (26)

As mentioned earlier, prostacyclin (PGI2) has important vasodilatory and antiplatelet properties, and acts locally to control blood flow and vascular tone. Unlike shear stress, *in vitro* studies have shown that cyclic strain in itself does not cause PGI2 secretion by EC. However, studies done by our laboratory have shown that cyclic strain enhances the synthetic ability of EC to produce PGI2 in the presence of exogenous arachidonic acid. (41) It has been suggested that the endogenous mechanism for arachidonate production is via cyclic strain mediated activation of the IP3 and DAG signal transduction pathways.

Endothelin-1, an endothelium-derived smooth muscle cell contracting factor, has been demonstrated to increase by 5 to 6-fold from basal levels with cyclic stretch. Thus physical forces exerted on ECs in culture can influence the secretion of this vasoconstrictive molecule. (42)

Smooth muscle cell migration and proliferation has been found to be affected by the effects caused by cyclic strain. These effects have been, in part, mediated by PDGF release. (43) Bovine aortic ECs were exposed to cyclic strain which resulted in a 2.6-fold increase in PDGF-B steady state mRNA. There is also accumulating evidence that links the release of vascular endothelial growth factor (VEGF) by vascular smooth muscle cells (VSMC) to normal endothelial cell (EC) function, repair and maintenance. An investigation carried out in our lab demonstrated the presence of secreted VEGF from VSMC by assaying the migration of EC. VEGF receptor phosphorylation on stretched EC was assayed by immunoblotting. The steady-state level of VEGF mRNA in stretched VSMC increased 3.3-fold above that of unstretched VSMC. EC migration was stimulated by media from unstretched and stretched VSMCs implicating that physiologic levels of stretch induces a biologically significant increase in VEGF secretion that provides an arterial stimulus for EC migration. (44)

6. INTRACELLULAR EFFECTS

As mentioned above, cyclic strain has been shown to cause upregulation of several different intracellular molecules including cell surface transducers, second messenger systems, and transcription factors. The function of IP3 and DAG as cell surface transducers is well described in biological systems. Cell surface receptors, stimulated by mechanical forces, initiate the hydrolysis of phosphatidylinositol 4,5 bisphosphate which produces IP3 and DAG. (45) These molecules then act on downstream targets. Specifically, DAG serves to activate protein kinase C, which itself has several targets, including MAP kinases. DAG also is involved in the synthesis of arachidonate, which is a precursor in the synthesis of PGI2. IP3 has been shown to modulate cellular calcium levels, which affect SMC contractility and vascular tone. (46)

The family of MAP kinases has multiple related proteins, including extra-cellular signal activated protein kinase (ERK), stress activated protein kinase (SAPK), and p38. MAP kinases are regulated by the phosphorylation of tyrosine and threonine residues, and have been shown to be activated by a variety of mitogenic stimuli, including shear stress and cyclic strain. Data from our lab suggests a role for MAP kinases in controlling cellular migration. Additional research has shown that MAP kinases act on nuclear transcription factors, such as nuclear factor kappa-B (NF-□b) and transcription activator protein-1(AP-1) in modulating cellular proliferation and cell cycle arrest. (47, 48) In this manner, it is thought that MAP kinase activity can have an effect on the migration and proliferation of vascular cells. In addition, the secretion of certain vasoactive substances, such as NO and PGI2, is mediated by MAP kinases. (49, 50)

Cyclic strain causes activation of several different MAP kinases including ERK, JNK and p38. Recent work in our laboratory with BAEC has shown that shear stress induces a more rapid and robust activation of ERK and p38 as compared with cyclic strain. The time course difference would suggest that there are different mechanoreceptors or alternative coupling pathways to detect different mechanical forces. (51, 52)

Yano *et al.* showed a significant increase in tyrosine phosphorylation of $pp125^{FAK}$ in EC by 30 minutes (3.4-fold) and 4 hours (5.9-fold) of exposure to cyclic strain. This was the first report of induction of $pp125^{FAK}$ by

Table 4. Effect of cyclic strain on transciption factors

	EFFECT OF CYCLIC STRAIN
AP-1	Activation of genes w/AP-1 binding sites
CRE	Activation of genes w/CRE binding sites
NF-κB	Activation of genes w/NF-κB binding sites
Egr-1	Activation of genes mediating MT1-MMP expression

a mechanical force. They showed that high strain induced a redistribution and reorganization of $pp125^{FAK}$, as well as F-actin and paxillin; the protein aligned with the long axes of ECs, which began to elongate after four hours exposure to strain. The data suggested that tyrosine phosphorylation of FAK (and paxillin) may regulate the reorganization of F-actin and the morphological changes induced by strain. (53)

P21-ras (ras), a small guanosine triphosphate (GTP)-binding protein, has been shown to play an important role in the signal transduction pathways to extracellular mitogenic stimulation. (54) It is a protein that is active in the GTP-bound state and inactive in the guanosine diphosphate (GDP)-bound state. Li *et al.* reported that ras is activated by shear stress in ECs maximally at 1 minute. (55) Cyclic strain also induced a transient activation of ras in a time-dependent manner (peak at 1 minute) in vascular endothelial cells. This study demonstrated for the first time that strain stimulates the conversion ratio to GTP-bound ras (from GDP-bound ras) and suggested the involvement of ras in the signal transduction pathway leading to strain-induced ERK 1 and 2 activation. (54)

pp60-src (src) is the best characterized protein tyrosine kinase and the prototype PTK of the cytosolic nonreceptor family. (56) It co-localizes with integrins within focal adhesions, supporting the assumption that mechanical signals are transduced into biomechanical information via chemical signaling molecules. (57) Schaller *et al.* showed an elevation in the phosphotyrosine content of FAK in $pp60^{v\text{-}src}$–transformed chicken embryo (CE) cells. They attributed this observation to certain possible scenario. First, it was possible that there was increased autophosphorylation due to FAK's activation by an, as yet, undefined mechanism. Second, $pp60^{v\text{-}src}$ may have activated an endogenous PTK, which subsequently phosphorylated FAK. Or third, it was possible that $pp60^{v\text{-}src}$ itself directly phosphorylated $pp125^{FAK}$. (58) Nevertheless, the fact that the autophosphorylation mutant FAK^{397} [phenylalanine substituted at tyrosine residue 397 (Tyr-397) of FAK] fails to form a complex with $pp60^{src}$ in src-transformed CE cells demonstrates that autophosphorylation at Tyr-397 is necessary to create the binding site for $pp60^{src}$.

Jalali *et al.* showed that src plays a critical role in the shear stress activation of MAP kinase pathways and induction of EC transcription factors. (59) Furthermore, the FAK-mediated association and activation of src was shown to be essential for maximal signaling to ERK 2. (60) Our data indicate that src is phosphorylated and activated with the application of stretch in EC in a time-dependent fashion. Peak phosphorylation of src occurs at 5 minutes, and peak activation occurs at 5 to 10 minutes. Further, our studies show that src is co-immunoprecipitated with FAK, implying that the two kinases interact directly. (61)

Cyclic strain has also been shown to activate transcription factors, thereby, regulating gene expression of certain cellular molecules (Table 4). Recent studies demonstrate that cyclic strain stimulates protein kinase C in (BAEC) as well as the induction of immediate early genes and the transcription factor activator protein-1 (AP-1) in human umbilical vein endothelial cells (HUVEC). Evidence for a heterogeneous response for growth, orientation and prostacyclin secretion has been obtained for a variety of EC exposed to cyclic strain. The results of our investigations demonstrate that EC exposure to cyclic strain leads to a significant induction of AP-1, CRE and NF-kB in HAEC and HUVEC, but not in BAEC. Furthermore, these findings are in marked contrast to the previously described shear stress induced activation of AP-1 and NF-kB in BAEC. There was also a temporal difference in their response such that stretch-induced activation of AP-1 and NF-kB peaked at 4 h, whereas CRE increased in a biphasic manner at 15 min and 24 h. (62) These results may partially explain the divergent effects of cyclic strain on EC gene expression and phenotype in EC from different vascular beds and species and underscore the difference in EC response to cyclic strain and shear stress.

There is a growing interest in the role of apoptosis, or programmed cell death, as a regulator of abnormal cell growth. In our lab, we examined whether Akt, a serine/threonine protein kinase known to promote cell survival by inhibiting apoptosis, is activated by cyclic strain in bovine aortic SMCs. Bovine aortic SMCs were cultured on flexible-bottomed membranes and then serum-starved for 24 to 36 hours. TUNEL assay was used to identify apoptosis. Akt phosphorylation was significantly increased over that of the negative control after 30 minutes of cyclic strain and in the control group. Cyclic strain did not increase the prevalence of apoptosis in SMCs over the control implying a pro-survival function for cyclic strain. (63) This experiment suggests that cyclic strain may actually induce arterial wall thickening by tipping the balance toward arterial SMC proliferation through the inhibition of apoptosis. Certainly, more work needs to be done to understand this process.

7. CONCLUSION

Atherosclerotic disease has risk factors which are systemic in nature, but its manifestations are specific, and localized to certain segments of the vascular tree. Hemodynamic forces are influenced by vessel and blood flow parameters, and vary throughout the vascular tree. Local hemodynamic forces affect the development and progression of atherosclerotic lesions. Studies using *in vitro* and *in vivo* models of the carotid bifurcation, coronary

arteries and the abdominal aorta have demonstrated specific areas of low shear stress and flow abnormalities. Other research has shown that blood pressure-induced arterial wall tension is manifest primarily at vessel branch points and bifurcations. In both models, hemodynamic abnormalities correlate with anatomic locations of atherosclerotic disease in humans.

Hemodynamic forces affect the progression of atherosclerotic disease at the cellular level by modulating the functional activity of EC and SMC. Cyclic strain causes activation of second messenger systems which are modulated by mechanical forces transduced by ECM proteins and integrin receptors. Cyclic strain also causes the secretion of vasoactive and thrombolytic substances which interact with multiple signal transduction pathways to influence vascular homeostasis, tone, blood flow, and, ultimately, the development of atherosclerotic disease. Understanding these mechanisms and their interplay with other cellular systems may ultimately lead to interventions that will halt or, even, reverse the atherosclerotic process.

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