Role of Reduced Endothelial Shear Stress in the Development of Atherosclerosis

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Summary

Atherosclerosis is a chronic, inflammatory, fibroproliferative disease primarily of large- and medium-sized conduit arteries. Although the entire vasculature is exposed to the atherogenic effects of the systemic risk factors, atherosclerotic lesions form at specific regions of the arterial tree, such as in the vicinity of branch points, the outer wall of bifurcations, and the inner wall of curvatures, where disturbed flow with low endothelial shear stress occurs. Vascular endothelial cells (ECs), uniquely situated at the interface between the blood and the vascular wall, are constantly exposed to fluid shear stress. Shear stress is reduced (<5 dynes/cm^2) at the outer walls of a bifurcation, branching, inner curvature walls, and these are the sites susceptible to atherosclerosis, the sites where atherosclerotic plaques are predominantly located. Physiological levels of laminar shear stress modulate cellular signaling and ECs function and are protective against atherogenesis. ECs are effective biological mechanotransducers which convert physical stimuli of shear forces to intracellular biochemical signals that provide normal vascular function and atheroprotection.Low endothelial shear stress attenuates nitric oxide (NO)-dependent atheroprotection; promotes low-density lipoprotein cholesterol (LDL) uptake, synthesis, and permeability; it promotes oxidative stress and inflammation, as well as vascular smooth muscle cell (VSMC) migration, differentiation, and proliferation; it attenuatesdegradation of extracellular matrix (ECM) in vascular wall, and plays potential role in atherosclerotic plaque neovascularization; low endothelial shear stress increases plaque calcification and thrombogenecity. The present article reviews the mechanisms of abovementioned changes in vascular walls caused by low endothelial shear stress.

Abbreviations: *NO- nitric oxide, ROS - reactive oxygen species , Ac-LDL- acylated-low-density lipoproteins , ECM - extracellular matrix , TNF- Cytokine tumor necrosis factor , VCAM- vascular cell adhesion molecule. MCP- monocyte chemoattractant protein*

Key words: *atherosclerosis; endothelial cell; endothelial shear stress; nuclear factor-kappa B; nitric oxide; endothelial nitric oxide synthase*

The build up of atherosclerotic plaques within the arterial tree is the underlying cause of most forms of cardiovascular disease, including coronary artery disease and stroke. Atherosclerosis remains a major cause of morbidity and mortality worldwide, and a thorough understanding of the underlying pathophysiological mechanisms is crucial for the development of new therapeutic strategies.Atherosclerosis is a multifactorial disease involving a complex array of contributing factors.Vascular endothelium has a key role in determining vascular function and is intimately involved in atherosclerosis. It handles important regulatory functions that include regulation of vascular tone, maintenance of the composition of subendothelial matrix, vascular smooth muscle cell proliferation, coagulation, fibrinolysis, permeability of lipoproteins and plasma proteins, and adhesion and migration of blood cells.Endothelial dysfunction (ED), which is characterized by an imbalance between relaxing and contracting factors, procoagulant and anticoagulant substances, and between pro-inflammatory and anti-inflammatory mediators, may play a particularly significant role in the pathogenesis of atherosclerosis[1].

Biological differencesof atherosusceptible endothelium include increased endothelial permeability to plasma macromolecules, increased (but still very low) endothelial proliferation, and increased immuno-surveillance by monocytes that attach and migrate into the artery wall[2]. These site-specific functional differences do not result in progression to significant inflammation unless additional systemic risk factors (e.g. hypercholesterolemia; hypertension; diabetes; smoking stress) are also present. The atherosusceptible phenotypes, therefore, may be considered to be in a sensitized—pre-lesional state[3].

Atherosclerotic lesions are found unevenly distributed in the vasculature. Atherosclerotic plaques develop essentially in arterial branch points, bifurcations and curvatures, regions of arterial narrowing. The carotid bifurcation, coronary arteries, abdominal aorta, and the iliofemoral arteries are lesion prone while other arteries are spared.In the carotid artery, for instance, atherosclerotic plaques tend to develop in the outer wall of the vessel at the level of the bifurcation[4, 5] and in the coronary arteries - in segments with bifurcations, as well as along the inner wall of curves[6,7].

In comparison, straight segments of the arteries remain lesion-free. On the other hand, it is not clear why some plaques remain quiescent for many years, while others progress rapidly. Findings suggest that independently of systemic factors, the presence of local hemodynamic factors such as wall shear stress plays a major role in the generation, progression, and destabilization of atherosclerotic plaques.

The haemodynamic conditions inside blood vessels lead to the development of superficial stresses near the vessel walls, which can be divided into two categories; 1. circumferential stress dueto pulse pressure variation inside the vessel; 2. shear stress generated by the frictional force of flowing viscous blood,and acting tangentially to the cell surface of the endothelium[10].Vascular endothelial cells (ECs), uniquely situated at the interface between the blood and the vascular wall, are constantly exposed to fluid shear stress [11].Cell deformation in response to applied shear stress is expressed as strain and depends on the mechanical and structural properties of the cell. Endothelial cells are capable of altering their structure and mechanical properties resulting in the generation of intracellular stress, e.g., cytoskeletal reorganization in response to flow. Tension developed by the cytoskeleton is related to altered cellular metabolism of living endothelial cells and expression of differentiated properties of the endothelium[12].

Relatively long, straight, unbranched arteries provide favorable conditions for laminar flow, which has radially symmetric parabolic velocity profile regardless of the vessel radius, and experience relatively high shear stress (15 dynes/cm^2).Near arterial branches and curves, velocity profile of blood flow acquires asymmetric character and then becomes disturbed (turbulent) for certain segment of the vessel until the symmetric velocity profile is developed again. At that, shear stress is reduced (<5 dynes/cm^2) at the outer walls of a bifurcation, branching, inner curvature walls, and these are the sites susceptible to atherosclerosis, the sites where atherosclerotic plaques are usually located. A typical laminar flow area is located in the greater curvature area and is marked as a low probability region for lesion formation, which is also known as a high wall shear stress area. A disturbed flow area is the lesser curvature area where lesion formation is more prevalent[13] and is also indicated as a low wall shear stress area.

Physiological levels of laminar shear stress modulate cellular signaling and ECs function and are protective against atherogenesis. ECs are effective biological mechanotransducers which convertphysical stimuli of shear forces to intracellular biochemical signals. As the vascular interface with flow-mediated shear stresses, the arterial endothelium senses changes in local haemodynamic characteristics and responds by initiating acute changes in artery wall vasomotion and chronic structural remodeling [14].Regulatory *physiological* responses to endothelial shear stress ensure adjustments of the vascular system and facilitates development and growth. However, localized regions of highly disturbed arterial flow areassociated with metabolic stress in the endothelium that sensitizes the cells to local inflammatory changes that favour a *pathological* outcome, the initiation and development of atherosclerosis.

Mechanotransduction involves transmission of the stress throughout the cell via the cytoskeleton (refer to Fig. 3). Here the membrane molecules participate in two ways: *1*) to passively transfer the stress to the cytoskeleton in one part of the cell and *2*) to respond to cytoskeletal deformations at sites remote from the stimulus (Flow mediated mechanotransduction). This involves activation of intracellular signaling pathways through the mechanosensitive ion channels, in particular, shear stress-activated potassium channels in endothelial cells first identified by Olesen et al.[15].Activation of the channels results in hyperpolarization of the endothelial cells. A mechanosensory complex on the endothelial cell surface has been identified as an initial transducer of mechanical forces consisting of the adhesion molecules PECAM-1 (platelet endothelial cell adhesion molecule-1), which is the mechanosensor, and VE-cadherin and VEGFR-2 (vascular endothelial growth factor receptor-2), which, once activated, stimulate a host of downstream signaling pathways. Mechanotransduction via this trimolecular complex mediates integrin activation, which causes elongation and alignment of ECs in the direction of flow, characteristic of cells in high-shear, protected regions[16]. This is an adaptive response that redistributes and reduces the local mechanical load experienced by the cell, reducing subsequent injury, and is dependent on anchoring of endothelial cells to the extracellular matrix via integrins. High laminar shear stress induces rapid conformational activation of integrins leading to remodeling of endothelial attachment sites and increased binding to the extracellular matrix, triggering cytoskeletal rearrangement and cell alignment[17].In contrast, blood flow within vessel curvatures, branch points, and bifurcations is typically disturbed, with overall low shear stress. As a result, ECs do not align parallel to the vessel long axis and show a less polarized shape, so-called cobblestone morphology. Because the cells do not align with the general direction of flow, their topology exposes them to greater shear stress gradients across the length of the cell, and these areas are also more prone to atherosclerosis[18] , Within the carotid bifurcation, where atherosclerosis often develops, the flow separates, disrupting the laminar profile and producing disturbed streamlines[19].Intracellular calcium is also an important signaling molecule that mediates critical intracellular pathways after stimulation of endothelial cells by a variety of agonists[20].

An important rapid physiological consequence of hemodynamic force transduction is the acute regulation of arterial diameter. When flow increases, arteries dilate by endothelial-dependent nervous system-independent relaxation of smooth muscle cells. There is now good evidence that the mechanism involves the enhanced release of endothelial-derived relaxing factors (EDRF).In regions spared of atherosclerosis, the blood surges during the cardiac cycle at an increasing then decreasing velocity as the contraction decreases resulting in unsteady but unidirectional laminar flow that is atheroprotective. In contrast, atherosclerosis develops in spatially predictable regions within large elastic and muscular distributing arteries near branches and bifurcations—where changes of vessel cross-sectional area occur over short distances—or as blood flow attempts to follow the tight inner curvature of the aortic arch.

The principal component of EDRF is nitric oxide (NO) [21].Endothelial nitric oxide (NO) synthase (eNOS) is a key mediator of abovementioned atheroprotective effects.NO is an endogenous activator of the soluble form of guanylate cyclase.Endothelial cells in vitro respond to shear stress by stimulation of cGMP, the elevation of which is proportional to the intensity of the shear stress up to ~40 dyn/cm^2. The increase appears to be regulated by a flow-induced activation of soluble guanylate cyclase which, in turn, is mediated by autocrine production of NO. NO reduces endothelial permeability, inhibits platelet adhesion and aggregation, reduces leukocyte adherence, LDL uptake, inhibits vascular smooth muscle cell proliferation, while simultaneously promoting EC survival[22].eNOS is constitutively expressed at a basal level, and its activity is calcium/calmodulin dependent. The activation of eNOS by flow involves increase of Ca^{2+} and calmodulin binding. However, the mechanisms of shear stress transduction are unclear because of the large number of regulatory possibilities associated with the cofactors for activation of this enzyme. Steady laminar flow induces synthesis of NO that is dependent on shear stress magnitude in the range of 2–12 dyn/cm^2 and upregulates the level of eNOS mRNA. Physiologic pulsatile endothelial shear stress constitutes the most potent stimulus for continuous NO production by the endothelium, an effect that is regulated at either transcriptional level through upregulation of eNOS gene expression or at post-transcriptional level by eNOS protein phosphorylation and activation[23].In contrast, disturbed turbulent flow fails to upregulate eNOS mRNA, and NO release remains at basal levels[24].In arterial regions with disturbed flow, low endothelial shear stress reduces the bioavailability of NO by decreasing eNOS messenger ribonucleic acid (mRNA) and protein expression, thereby exposing the endothelium to the atherogenic effect of local and systemic risk factors.

Prostaglandin I_2, also known as prostacyclin, is another endothelial vasodilatory substanceand the most potent natural inhibitor of platelet aggregation. Its synthesis and release from endothelial cells occur not only in response to a number of agonists, but also in the presence of increased shear stress.Low endothelial shear stress downregulates prostacyclin, while upregulating endothelin-1 (ET-1)[25], a potent vasoconstrictive and mitogenic molecule, thereby precipitating atherosclerosis.

Low ESS increases plaque thrombogenecity by downregulating the expression of eNOS and prostacyclin, well known for their anti-thrombotic properties.

Normal shear stress promotes anti-oxidative and anti-inflammatory processes. Various studies have shown that the presence of a normal or increased wall shear stress (≥10 -15 dynes/cm²) has a protective effect on the endothelium mediated by inhibition of endothelial proliferation, a local anti-inflammatory effect, prevention of apoptosis of endothelial cells, and increased expression and activity of antioxidant enzymes (superoxide dismutase and nitric oxide synthase) in endothelial cells[26].

Cytokine tumor necrosis factor-(TNF-) is an important mediator of the inflammatory processes that occur during the progression of atherosclerosis[27]. Produced by macrophages that infiltrate the lesion,cytokines such as TNF- are known to induce the expression of many endothelial genes that contribute to the complex processes involved in atherogenesis.Well known examples include the transcriptional regulation of various adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-vascular cell adhesion molecule-1 (VCAM-1), and E-selectin[28].Studies of cultured endothelial cells have revealed that prolonged unidirectional high shear stress suppresses pro-inflammatory activation and leukocyte recruitment in response to such inflammatory stimulus as tumor necrosis factor-alpha (TNF-α)[29].This observation is supported by in vivo studies where endothelial cells at low shear, atherosusceptible sites express pro-inflammatory markers. In contrast, pro-inflammatory signaling is suppressed in regions of the arterial tree that are protected from atherosclerosis and exposed to high shear stress[30, 31].

Atherogenesis is promoted by decreased shear stress because it is associated with reduction in several vascular wall functions including 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[32].Observations suggest that shear stress acts through the endothelium to modulate smooth muscle cell gene expression providing an atheroprotective phenotype to the vessel wall. The uptake of acylated-low-density lipoproteins (Ac-LDL) is decreased in endothelial- smooth muscle cell cocultures exposed to laminar shear stress relative to static cultures[33].

The level of shear stress is inversely correlated with smooth muscle cell(SMC) density, whereas exposure to laminar flow does not influence cell density[34].These studies suggests 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.

Mechanisms by which high shear stress reduces pro-inflammatory signaling presumably involves two key pro-inflammatory signaling pathways: the mitogen-activated protein kinase (MAPK) pathway and nuclear factor-kappa-B (NF-κB) pathway[35]. The MAPKs are a group of serine/threonine protein kinases that play an important role in many cellular processes including apoptosis, proliferation, and inflammation. The transcription factor NF-κB has been closely linked with cardiovascular health and disease due to its control of multiple processes including immunity, inflammation, cell survival, differentiation and proliferation, and regulation of cellular responses to stress, hypoxia, stretch, and ischemia. NF-κB signaling in endothelial cells typically leads to the expression of genes that induce recruitment of inflammatory cells to the vessel wall, including ICAM-1 (intercellular adhesion molecule-1), VCAM-1, E-selectin, and cytokines such as TNF-α and IL-1[36].Studies in mice and pigs revealed that the expression of NF-κB subunits is significantly increased in regions of the aorta exposed to low shear stress compared to high shear stress[37]. Cells exposed to low shear stress are primed for activation by a pro-inflammatory stimulus due to an increased expression of NF-κB subunits[38].Low shear stressinduced NF-κB activation upregulateschemoattractant chemokines, such as monocyte chemoattractant protein (MCP)-1; and pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, and interferon(IFN)-γ[39]. Presumably high laminar shear stress suppresses inflammation in part by modulating or inhibiting NF-κB and MAPK signaling.

Low endothelial shear stress promotes oxidative stress. Low endothelial shear stress promotes production of reactive oxygen species (ROS) into the intima and, eventually, oxidation of low density lipoproteins (LDL), by enhancing gene expression and post-transcriptional activity of the major oxidative enzymes (nicotinamide adenine dinucleotide phosphate [NADPH] oxidase and xanthine oxidase) at EC membranes. Low shear stress appears also to downregulate the intracellular ROS scavengers, such as manganese superoxide dismutase and glutathione, further augmenting local oxidative stress. Generated ROS degrade NO and its co-factors, reducing the bioavailability of atheroprotective NO and further enhancing the production of ROS (e.g., superoxide [O_2^-] or peroxynitrite [$ONOO^-$])[40]. Overproduction of ROSis an integral part of the development of atherosclerosis. Oxidative stress participates in pro-atherogenic mechanisms of endothelial dysfunction[41].

Neovascularization (angiogenesis) constitutes a key factor in the progression and vulnerability of atherosclerotic plaques by supplying them with lipoproteins, inflammatory cells, matrix proteases, and ROS (99). Low endothelial shear stress indirectly promotes intimal neovascularization by inducing intimal thickening and thus ischemia, upregulating the expression of VEGF and other angiogenic factors (e.g., angiopoietin-2), enhancing local inflammation, oxidative stress, and expression of matrix degrading enzymes and accentuating EC and VSMC migration and proliferation[42].

Low and disturbed flow causes intensive degradation of extracellular matrix (ECM) of ECs and attenuates ECM synthesis. Interferon-γ, a pro-inflammatory cytokine derived by the activated T-lymphocytes in response to low endothelial shear stress, constitutes a potent inhibitor of collagen synthesis by VSMCs and simultaneously promotes Fas-related VSMC apoptosis[43]. Vascular smooth muscle cell apoptosis can be also induced by low shear-generated oxidative stress through activation of Fas signaling pathways [44]. Next to its role in VSMC turnover, NO constitutes potent inducer of collagen synthesis by VSMCs as well as anti-inflammatory molecules. Downregulated endothelial expression of eNOS genes due to low endothelial shear stress might contribute to increased inflammation and reduced matrix synthesis.

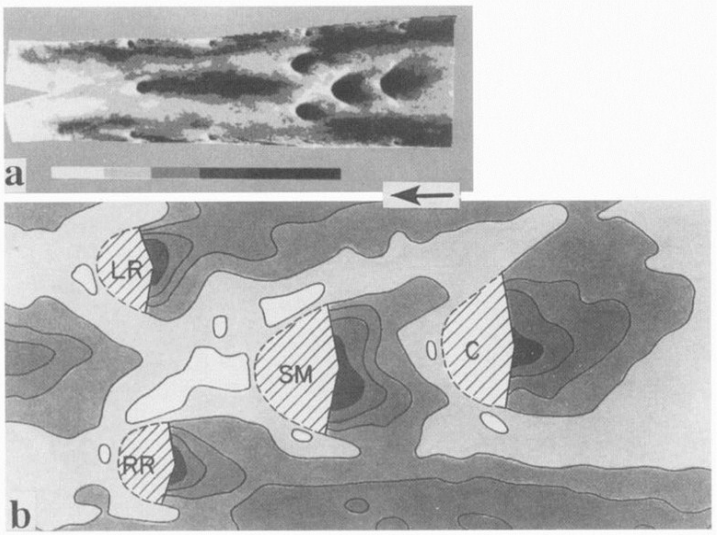

Fig. 1 Localization of human atherosclerosis in abdominal aorta. *a*: it demonstrates lesions associated with regions of predicted complex hemodynamic profiles near branch arteries. *b*: detail of *a* showing distribution near right and left renal arteries (RR, LR) as well as superior mesenteric (SM) and celiac branches (C). Arrow indicates overall direction of flow.

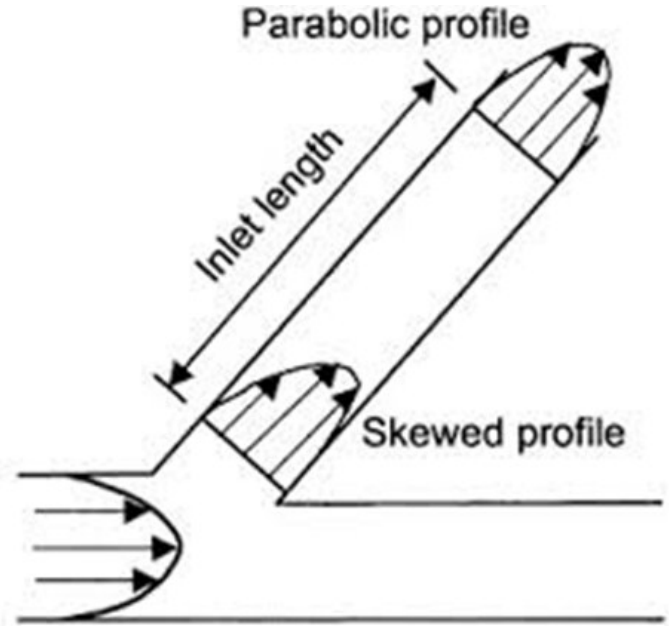

Fig. 2. Flow entering a side branch results in skewed profile; it takes a certain entrance length before the parabolic velocity profile is developed again.

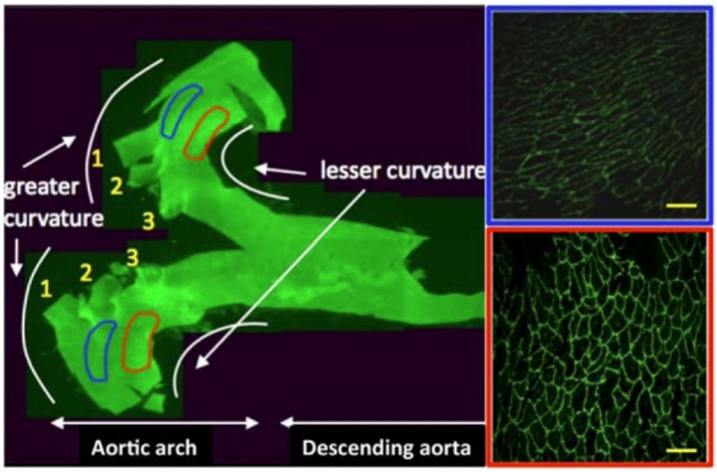

Fig. 3.En Face immunohistochemistry. The greater curvature of the aortic arch is exposed to steady laminar flow and is protected from atherosclerosis. Regions of smaller curvature and side branches indicated by numbers (1, 2, and 3) are exposed to disturbed flow and are athero-prone areas. The aorta was prepared from a 7 weeks old C57BL/6 wild type mouse.

Conclusion:

Local hemodynamic factors influence the evolution of atherosclerotic disease and may contribute to explaining the differences in distribution and progression of different atherosclerotic plaques. The presence of a low wall shear stress favors progression of the plaque, while a physiologic shear stress has a protective effect in the vascular endothelium. In the atheroprone regions, the endothelium has a pro-inflammatory phenotype associated with low nitric oxide production, reduced barrier function and increased proadhesive, pro-coagulant and proproliferative properties. Athero-resistant regions are exposed to laminar flow and high shear stress that induce pro-survival antioxidant signals and maintain the quiescent phenotype in ECs. Indeed, various flow patterns contribute to phenotypic and functional heterogeneity of arterial endothelium whose response to pro-atherogenic stimuli is differentiated. This may explain the preferential development of endothelial dysfunction in arterial sites with disturbed flow.

Thus low shear stress provides predisposition for atherogenic transformation by contributing to endothelial dysfunction whereas high shear areas might shield against atherosclerosis by enhancing endothelial protection. Shear stress of laminar blood flow is a critical factor in maintaining normal physiologic vascular function including thromboresistance, barrier function and vascular homeostasis.

Further studies are to be conducted to shed light on the mechanisms by which shear stress modulates endothelial apoptosis, proliferation, and thrombogenicity, all of which contribute to the pathogenesis of atherosclerosis. Better understanding of these mechanisms will enable targeted therapeutic interventions that promote a protective phenotype in atherosusceptible regions in order to slow or even halt the progression of cardiovascular disease.

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