Endothelium and haemorheology

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Summary. The vascular endothelium has been recognized to have a central importance in maintaining vascular homeostasis and in preventing cardiovascular disease. The mechanisms underlying the regulation of its function are extremely complex, and are principally determined by physical forces imposed on the endothelium by the flowing blood. In the present paper, we describe the interactions between the rheological properties of blood and the vascular endothelium. The role of shear stress, viscosity, cell-cell interactions, as well as the molecular mechanisms that are important for the transduction of these signals are discussed both in physiology and in pathology, with a particular attention to the role of reactive oxygen species. In the final conclusions, we propose an hypothesis regarding the implications of changes in blood viscosity, and particularly on the significance of secondary hyperviscosity syndromes.

Key words: endothelium, haemorheology, viscosity.

Riassunto *(Endotelio ed emoreologia)*. All'endotelio vascolare è stato recentemente riconosciuto un ruolo cruciale nel mantenere l'omeostasi vascolare e nel prevenire la genesi delle patologie cardiovascolari. I meccanismi alla base della regolazione della cosiddetta funzione endoteliale sono estremamente complessi, e sono principalmente legati alla interazione tra endotelio e lo stress meccanico imposto dal flusso ematico. In questo articolo, descriviamo i meccanismi di questa interazione tra le proprietà fisiche e reologiche del sangue e l'endotelio. Il ruolo di shear stress, viscosità, interazioni cellula-cellula, ed i meccanismi molecolari di questi fenomeni sono discussi in condizioni fisiologiche e patologiche, con un'attenzione particolare al ruolo dei radicali liberi dell'ossigeno. Nelle conclusioni finali, proponiamo un'ipotesi riguardo alle implicazioni delle modificazioni nella viscosità ematica, particolarmente per quello che riguarda le sindromi da iperviscosità secondaria.

Parole chiave: endotelio, emoreologia, viscosità.

INTRODUCTION

The endothelium layer covers the inner surface of the whole vascular system. This monocellular layer separates all tissues from the circulating blood [1]. While in the past it as been considered to be an inert physical barrier, acting only as a selective sieve to facilitate bidirectional passage of macromolecules and blood gases between tissues and blood, research lines in the '80ies and '90ies have clearly demonstrated that the endothelium is a dynamic organ which holds a leading role in regulating vascular homeostasis.

Because its peculiar location allows it to sense changes in haemodynamic forces and blood-borne signals, the endothelium exerts its function in maintaining vascular homeostasis through the balanced release of a number of autocrine and paracrine substances in response to physical, biological and chemical stimuli. Substances released from the endothelium regulate thrombosis, thrombolysis, platelet adherence, vascular tone, lipid metabolism and inflammation *(Figure 1)*. Given the critical role of these mechanisms, the disruption of the endothelial balance, a phenomenon called endothelial dysfunction, is a precursor of the pathogenesis of many diseases including atherosclerosis, hypertension, sepsis and some inflammatory syndromes [2].

In the following paragraphs, we will describe the relation between endothelium and hemorheology, and how a dysfunction in this relationship can interfere with the production of endothelial autacoids and vascular flow.

NOTIONS OF ANATOMY, PHYSIOLOGY AND PATHOPHYSIOLOGY OF THE VASCULAR ENDOTHELIUM

While its anatomical structure is extremely simple, composed as it is by a single layer of mesenchymal cells, the endothelium is an extremely complex tissue from the metabolic point of view. Of interest, while endothelial cells are (at rest) flat, most of the thickness of the endothelium (up to several hundred nms) is determined by a dynamic structure lying on its luminal surface. This structure, denominated the endothelial surface layer (ESL, *Figure 2*) is composed of proteins, glycolipids, glycoproteins and glycosaminoglycans. The molecular domains hosted in this glycocalyx function as receptors for adhesion molecules, components of the coagulation/fibrinolysis system, transporter for oxygen and macromolecules, and, most importantly, as mechanical transducers of the physical stress deter-

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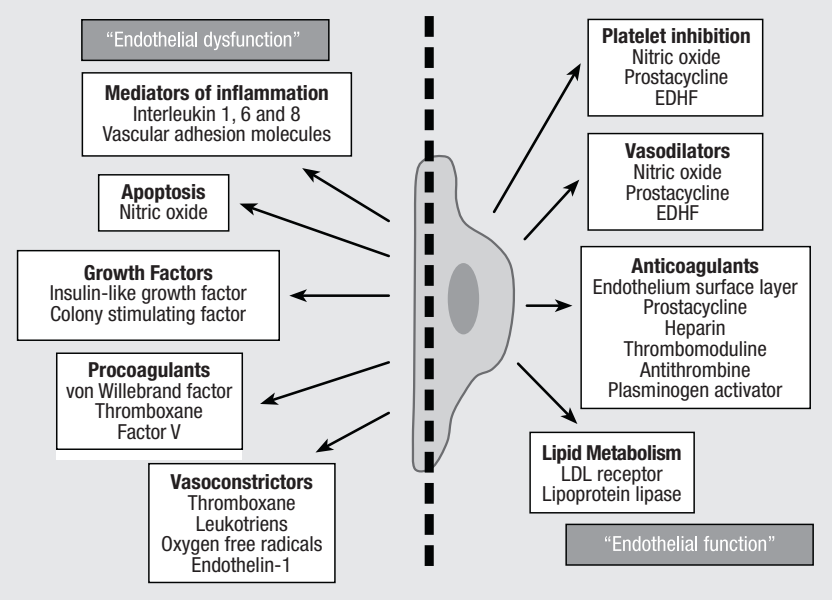

Fig. 1 | *Endothelial "function" (*i.e.*, the production of protective autacoids by the vascular endothelium) and "dysfunction" (*i.e.*, the involvement of the endothelium in vascular pathology). EDHF: Endothelium-Derived Hyperpolarizing Factor; LDL: Low-Density Lipoprotein.*

mined by the flowing blood on the surface of the endothelium. With its thickness, the ESL occupies a large fraction of the lumen in capillaries and arterioles, and it has been shown that vascular resistance measured at the level of microvessels (where the ratio of ESL thickness to vascular lumen is highest) is much higher as compared to the resistance measured in glass capillaries having the same diameter [3]. This increase in vascular resistance determined by the ESL depends on: 1) physical reduction of the vascular lumen by the ESL 2) electrochemical interaction between ESL and blood components, which increases friction forces [4].

As said, the ESL functions as a transducer of mechanical forces: the modifications imposed by shear stress, *i.e.*, the friction force determined by the flowing blood that acts tangentially on the ESL, determine mechanical modifications of the intracellular cytoskeleton, which is, on one side, structurally bound to the ESL, and, on the other, to several stretch-activated sensors, mostly protein G systems and ion channels. It seems that, in this molecular cascade, activation of MAP kinases plays a central role. Indeed, these ubiquitously expressed serine/threonine protein kinases (which are involved in the regulation of cell growth, transformation and differentiation), and in particular the extracellular signal–regulated kinases (ERK1/2)), activate several enzymes which include protein kinases (p90rsk, MAPKAP, Raf-1, MEK), transcription factors (c-myc, c-jun, c-fos, p62TCF), and cell surface proteins (EGF receptor) [5]. The cascade of molecular events that follows these reactions regulates the production of endothelial autacoids, and in particular the synthesis of nitric oxide (NO) [6], as discussed below.

Thanks to these mechanisms, early upon detection of increases in shear stress, rapid changes in ionic conductance, inositol triphosphate production and cytosolic Ca^{2+} concentrations can be observed in the endothelial cell. Opening of K^+ channels facilitates membrane hyperpolarization, which provides an electrochemical gradient for Ca^{2+} entry. The plasma membrane thickens and starts to form invaginations that are named caveolae [7], where the synthesis of NO occurs, stimulated by the increased Ca^{2+} availability. NO is a highly reactive free radical [8] with a number of effects, among which a potent influence on haemorheology [9]. Indeed, NO increases red blood cell and platelet deformability, reduces platelet adhesion and aggregation [10], reduces leukocyte adhesion [11], reduces endothelial expression of adhesion molecules (which, although not being an intrinsic characteristic of blood, is an important

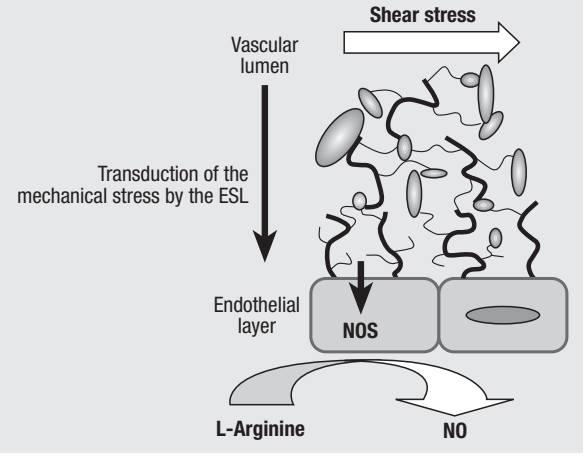

Fig. 2 | *Endothelial production of nitric oxide (NO) is stimulated by oscillatory shear stress, transmitted by the endothelial surface layer to the endothelial cells.*
NO: Nitric Oxide; NOS: Nitrous Oxide Systems; ESL: Endothelial Surface Layer

determinant of blood-vessel interactions) [12] and, most importantly, it causes vasodilation [13].

While these changes are induced acutely by shear stress, and in particular by oscillatory shear stress [14], in cases where this physical stimulus is maintained for prolonged periods, genomic induction ensues, probably mediated by activation of the nuclear factor kB (NFkB) transcriptional factor. Because NFkB binding sites are found in the promoter regions of a variety of genes, this system might have a particular importance in altering gene expression in response to sustained variations in shear stress. In particular, the increased expression of the endothelial enzymes NO synthase, one of the effects of sustained increases in shear stress, explains the parallel sustained increase in the production of this free radical [15]. Considering the beneficial effects of NO in vascular physiology, one can safely assume that the benefit associated with physical exercise (*i.e.*, chronic increase in oscillatory shear stress) in cardiovascular patients is indeed mediated by the above described mechanisms [16].

In this scenario, a particular importance has been given to other free radicals, the reactive oxygen species [17] (ROS). The ROS are free radicals normally produced in low concentrations by the mitochondrial respiratory chain and normally scavenged by multiple intra- and extracellular mechanisms, including the enzyme superoxide dismutase, glutathione and vitamin C. When produced in supranormal concentrations, ROS can overcome these scavenging mechanisms and rapidly react with NO to form the highly toxic peroxynitrite [18]. This may reduce the bioavailability of endothelium-derived NO, impairing its vasodilator activity, and, possibly, directly counteract NO-induced protective effects, as ROS cause vasoconstriction and vascular damage [19]. Therefore, these high concentrations of ROS and peroxynitrite are potent toxics for cellular structures, due to their capacity to oxidize and damage or inactivate a variety of cellular structures. Interestingly, the redox state of endothelial cells was found to be dependent on the type of the shear stress applied, an observation which provides an interesting mechanistic clue to the phenomena described until now: it has been shown that oscillatory and steady (low-grade) laminar shear stress differentially affect human endothelial redox state, the latter causing induction of ROS-producing NADH oxidase [14]. Downstream to reduced NO bioavailability and (corresponding) increased ROS bioavailability, potential mechanisms that have been proposed to explain the REDOX-dependency of vascular homeostasis include increased LDL uptake, accumulation of inflammatory cells (a process that could be emphasized by the increased expression of ligands such as ICAM and VCAM). Finally, pulsatile shear stress downregulates the expression of the gene encoding for endothelin-1, a potent vasoconstrictor and a trigger (in a feed-forward mechanism) of ROS formation [20]. In sum, steady, low-grade shear stress (and/or disruptions in the transduction mechanism of shear stress, *i.e* the ESL) cause increased ROS production. In this apparently simple mechanism lies the pathophysiology of most cardiovascular syndromes, and the importance of ROS production as the common pathway of vascular pathobiology cannot be overstated, as discussed in more detail elsewhere [17, 21].

Physiological shear stress levels have been demonstrated to induce, in vitro, atheroprotective endothelial gene expression patterns, while a low-grade shear stress level was associated with the expression of an atherogenic phenotype [22]. To this regard, several studies, a few decades ago, have shown that changes in the "quantity" (*i.e.*, both increases and decreases) as well as in the "quality" (from oscillatory, laminar to steady, turbulent) of shear stress are the most likely explanation for the evidence that atherosclerosis tends to develop preferentially at vascular bifurcations [23, 24]. Taken together, these phenomena provide a background rationale to why atherosclerotic lesions preferentially originate in areas of disturbed flow associated with low – non oscillatory, non laminar – shear stress [25].

WHAT DETERMINES SHEAR STRESS

The mechanical forces determined by vascular hemodynamics on the vasculature act along two gradients: a circumferential one, associated with variations in pulse pressure in the vascular lumen, and a longitudinal one, *i.e.* shear stress, which is the force that contrasts the friction applied to the blood by the vascular wall. Blood flow in arteries, arterioles and capillaries causes a degree of shear stresses in the range of 0-50 dyn/cm^2, according to the site and the anatomy of the vessel [26]. Obviously, important determinants of shear stress are geometrical (bifurcations, aneurysms, tortuosity of the vessel), biological (mainly NO release) and systemic (blood pressure) factors. In less plain terms, the two components of shear stress are wall shear rate and blood viscosity, where shear rate is the rate at which adjacent layers of fluid move with respect to each other. When one considers the fundamental assumption of fluid mechanics that the velocity of a fluid upon a surface nears zero, shear rate can be understood as the gradient of blood flow velocity between the vascular wall and the peak velocity located somewhere close to the middle of the vessel (in cylindrical vessels). The second component of shear rate is blood viscosity. While viscosity is normally understood as an intrinsic property of a fluid (essentially its capacity to offer resistance to flow), blood viscosity is influenced by several factors, among which of obvious importance are blood cell deformability [27], expression of adhesion molecules etc. As said above, while endothelium-derived autacoids modify both shear rate (by modulating vascular tone) and blood viscosity, in turn, the interaction between shear rate and blood viscosity is a critical modulator of endothelial function, and, consequently, of vascular homeostasis. For instance, studies employing blood substitutes have clearly shown that an elevated viscosity elicits a vasodilatory response due to increased shear stresses [28].

In sum, shear rate, (hematocrit) and viscosity concur to determine shear stress and, through the endothelial cell's biochemical apparatus, regulate vascular homeostasis. The next paragraph will discuss how changes in viscosity alter this equilibrium.

BLOOD HYPERVISCOSITY AND ITS EFFECTS ON ENDOTHELIAL FUNCTION

According to the 1970 Wells' classification, hyperviscosity syndromes are divided into three forms:

- polycytemic syndromes, which are the resultant of an increase in the number of circulating blood cells, which can be demonstrated by changes in hematocrit counts;
- sclerocytemic syndromes, where an altered deformability of cellular membranes determines the decreased fluidity of the blood;
- syndromes associated with an increased serum viscosity. In these syndromes, an altered concentration and/or specific properties of an abnormally produced plasma protein (for instance, paraproteinemias) determine increased blood viscosity.

In order to make some examples, syndromes associated with "primary hyperviscosity" include polycythaemias, acute and chronic leukemias, reactive leukocytosis, thrombocytosis, thrombocythaemia and platelet hyperactivity, cryoglobulinemia as well as hyperfibrinogenaemia and myeloma.

In terms of the effects of these changes in blood viscosity on endothelial function, several lines of evidence demonstrate that hyperviscosity causes, as discussed above, a worsened endothelial function and patient prognosis. For instance, in the case of sickle cell disease, vasoocclusive crises due to enhanced adhesion of blood cells to the vascular endothelium as well as abnormal vasomotor tone regulation are a characteristic manifestation and a very common cause of morbidity and mortality. Confirming a deleterious effect of pathologically increased viscosity on endothelial function, despite the increased wall shear stress (due to the increase in flow and in viscosity), patients with sickle cell disease have normal resting brachial artery diameters and a markedly blunted flow-mediated dilation (a parameter of endothelial function) [29]. In sum, primary hyperviscosity syndromes compromise the mechanisms responsible for the transduction of the endothelium-dependent vasodilator signal, causing impaired endothelial responsiveness to changes in shear stress due to the chronically increased wall shear stress in these patients [29]. Taken together, these considerations provide a mechanistic insight for the observation that abnormal blood viscosity is associated with markers of systemic atherogenesis such as intima-media thickness [30].

THE CASE OF SECONDARY BLOOD HYPERVISCOSITY SYNDROMES – NOT SO BAD?

Along with the primary hyperviscosity syndromes, several other conditions have been shown to be associated with an increased plasma viscosity. Among these, are cardiac, peripheral and cerebral ischemia [31], as shown in Raynaud's syndrome (in which the viscosity of the blood refluent from ischemic territories is higher than that in the contralateral arm) [32], peripheral arterial disease (where blood viscosity appears to be linearly correlated with Fontaine stage), carotid atherosclerosis [33], cardiac ischemia, where our group showed that blood viscosity increases in patients who develop ischemia during exercise testing and during atrial pacing [34]. More in general, blood viscosity is increased in the presence of cardiovascular risk factors [35]. Based on these observations, one can classify hyperviscosity in primary forms, where hyperviscosity is the mechanism of disease (Wells' classification), and secondary forms, where hyperviscosity is actually caused by (or at least associated with), ischemia. Since this subclassification was introduced [36], and based on the considerations made above, it is now known that activation of the ischemic endothelium leads to a series of molecular events that cause changes in blood viscosity [37]. In an example of the importance of endothelial function, blood viscosity was observed to be significantly increased in the morning hours (*i.e.,* when ischemic events are most likely to occur) in patients with risk factors for and/or chronic cardiovascular disease, even in the absence of ongoing ischemia [38-40].

Several lines of evidence confirm this association between ischemia and determinants of blood viscosity: for instance, patients with myocardial infarction show a decreased erythrocyte filtration and an increased blood viscosity, which are accompanied by an increased rigidity of the erythrocyte membrane [41]; in animals, these changes are associated with an increased production of ROS by membrane NADH oxidase [42]. In sum, red blood cell deformability and blood viscosity appear to be particularly REDOX sensitive [43], an observation that confirms the critical importance of ROS in vascular pathophysiology. In sum, there are conditions where hyperviscosity is a consequence (not a cause, as in the primary syndromes) of vascular disease. The significance of ischemia-induced hyperviscosity is described below.

While it is commonly accepted that sustained (primary or secondary) hyperviscosity is a source of further ischemia [44, 45], in an effort to understand the true "meaning" of ischemia-induced hyperviscosity an important consideration needs to be done. The increased viscosity observed in coronary artery disease and/or peripheral arteriopathy has been traditionally interpreted as a consequence or ROS-mediated damage to blood cells and endothelial membranes. However, one has to see the other side of the coin: an increased viscosity, might, at the beginning, act to increase shear stress in the endothelial microenvironment *(Figure 3)*. As discussed above, this might increase NO release, triggering the antiatherosclerothic genotype described above (paragraphs 1 and 2). As well, a reduced deformability of red blood cells might increase their permanence within microvessels, favouring oxygen extraction and tissue perfusion. In other words, haemorheological changes of secondary syndromes might be an important com-

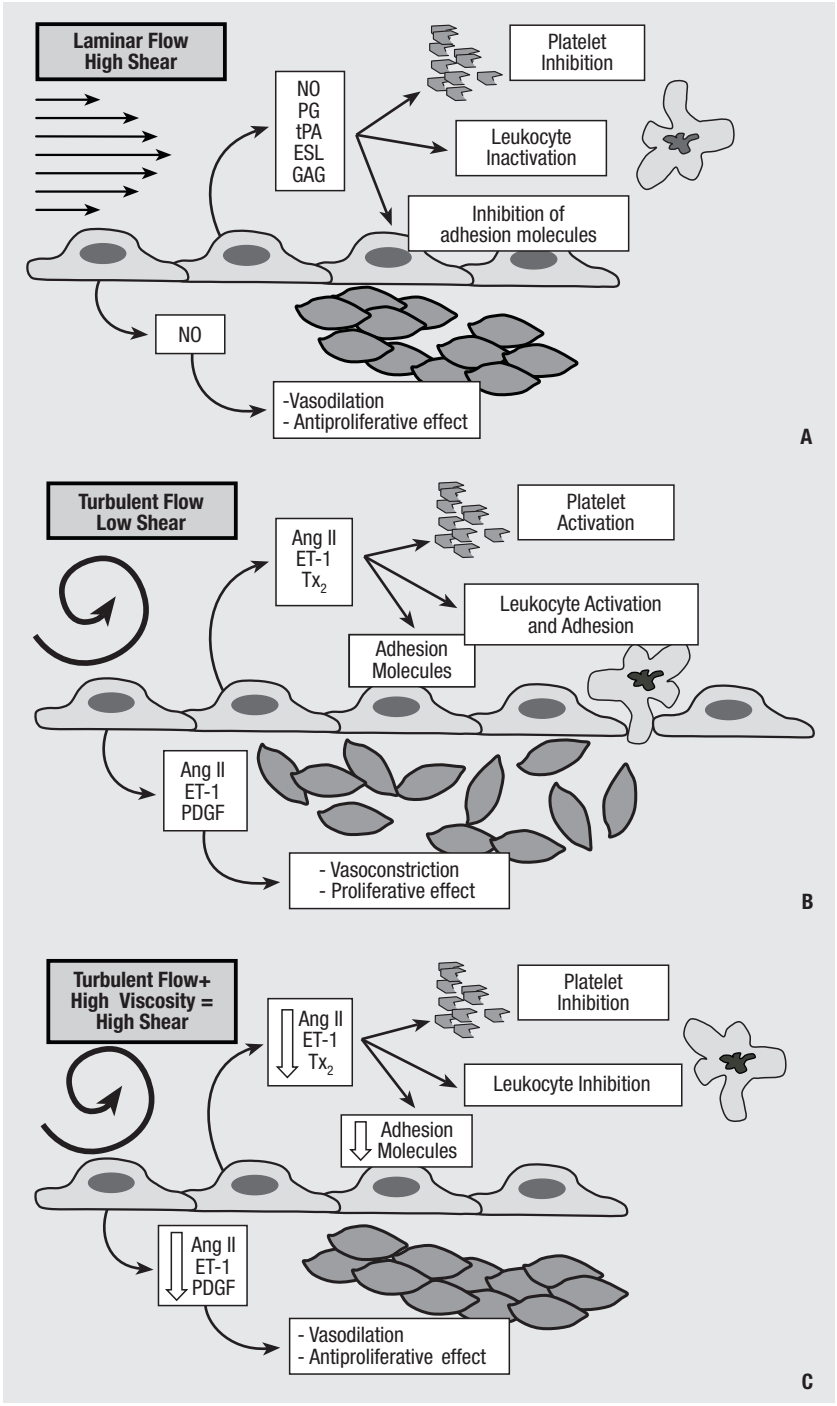

Fig. 3 | *Panels A-C: In normal conditions, laminar flow determines high shear stress, which induces a protective endothelial phenotype. In conditions of turbulent flow, this shear stress is reduced, causing the endothelium to loose it protective effect. In this scenario, secondary hyperviscosity might represent a physiological counterregulatory phenomenon aimed at increasing shear stress and endothelial physiology despite non-laminary flow.*
NO: nitric oxide; PG: Prostaglandin; tPA: tissue Plasminogen Activator; ESL: endothelial surface layer; GAG: glycosaminoglycan; Ang II: Angiotensin II; ET-1:Endothelin-1; PDGF: Platelet-derived growth factor; Tx_2: Thromboxane 2.

pensatory mechanisms aimed at normalizing vascular homeostasis. An excess of this compensatory mechanism might produce the opposite effects, as persistent hyperviscosity will lead to impaired perfusion and further ischemia. In conclusion, secondary hyperviscosity might be one of the many (*e.g.*, immunity) compensatory systems which, when gone awry, actually become source of disease.

References

1. Galley HF, Webster NR. Physiology of the endothelium. *Br J Anaesth* 2004;93:105-13.
2. Verma S, Buchanan MR, Anderson TJ. Endothelial function testing as a biomarker of vascular disease. *Circulation* 2003;108:2054-59.
3. Pries AR, Secomb TW. Microvascular blood viscosity in vivo and the endothelial surface layer. *Am J Physiol Heart Circ Physiol* 2005;289:H2657-64.
4. Pries AR, Secomb TW, Gaehtgens P. The endothelial surface layer. *Pflugers Arch* 2000;440:653-66.

5. Traub O, Berk BC. Laminar shear stress: mechanisms by which endothelial cells transduce an atheroprotective force. *Arterioscler Thromb Vasc Biol* 1998;18:677-85.
6. Weinbaum S, Zhang X, Han Y, Vink H, Cowin SC. Mechanotransduction and flow across the endothelial glycocalyx. *Proc Natl Acad Sci USA* 2003;100:7988-95.
7. Masuda H, Kawamura K, Nanjo H, Sho E, Komatsu M, Sugiyama T, Sugita A, Asari Y, Kobayashi M, Ebina T, Hoshi N, Singh TM, Xu C, Zarins CK Ultrastructure of endothelial cells under flow alteration. *Microsc Res Tech* 2003; 60:2-12.
8. Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. *Biochem J* 2001;357:593-615.
9. Celermajer DS. Endothelial dysfunction: does it matter? Is it reversible? *J Am Coll Cardiol* 1997;30:325-33.
10. Radomski MW, Vallance P, Whitley G, Foxwell N, Moncada S. Platelet adhesion to human vascular endothelium is modulated by constitutive and cytokine induced nitric oxide. *Cardiovasc Res* 1993;27:1380-2.
11. Godin C, Caprani A, Dufaux J, Flaud P. Interactions between neutrophils and endothelial cells. *J Cell Sci* 1993;106 (Pt 2):441-51.
12. Naseem KM. The role of nitric oxide in cardiovascular diseases. *Mol Aspects Med* 2005;26:33-65.
13. Furchgott RF, Carvalho MH, Khan MT, Matsunaga K. Evidence for endothelium-dependent vasodilation of resistance vessels by acetylcholine. *Blood Vessels* 1987;24:145-9.
14. De Keulenaer GW, Chappell DC, Ishizaka N Nerem RM, Alexander RW, Griendling KK. Oscillatory and steady laminar shear stress differentially affect human endothelial redox state: role of a superoxide-producing NADH oxidase. *Circ Res* 1998;82:1094-101.
15. Uematsu M, Ohara Y, Navas JP, Nishida K, Murphy TJ, Alexander RW, Nerem RM, Harrison DG. Regulation of endothelial cell nitric oxide synthase mRNA expression by shear stress. *Am J Physiol* 1995;269:C1371-8.
16. Hambrecht R, Wolf A, Gielen S, Linke A, Hofer J, Erbs S., Schoene N, Schuler G. Effect of exercise on coronary endothelial function in patients with coronary artery disease. *N Engl J Med* 2000;342:454-60.
17. Kojda G, Harrison D. Interactions between NO and reactive oxygen species: pathophysiological importance in atherosclerosis, hypertension, diabetes and heart failure. *Cardiovasc Res* 1999;43:562-71.
18. Huie RE, Padmaja S. The reaction of no with superoxide. *Free Radic Res Commun* 1993;18:195-9.
19. Elliott SJ, Lacey DJ, Chilian WM, Brzezinska AK. Peroxynitrite is a contractile agonist of cerebral artery smooth muscle cells. *Am J Physiol* 1998;275:H1585-91.
20. Malek AM, Greene AL, Izumo S. Regulation of endothelin 1 gene by fluid shear stress is transcriptionally mediated and independent of protein kinase C and cAMP. *Proc Natl Acad Sci USA* 1993;90:5999-6003.
21. Gori T, Forconi S. The role of reactive free radicals in ischemic preconditioning-clinical and evolutionary implications. *Clin Hemorheol Microcirc* 2005;33:19-28.
22. Malek AM, Alper SL, Izumo S. Hemodynamic shear stress and its role in atherosclerosis. *JAMA* 1999;282:2035-42.
23. Gnasso A, Irace C, Carallo C, De Franceschi MS, Motti C, Mattioli Pl Pujia A. In vivo association between low wall shear stress and plaque in subjects with asymmetrical carotid atherosclerosis. *Stroke*. 1997;28:993-8.
24. Giddens DP, Zarins CK, Glagov S. The role of fluid mechanics in the localization and detection of atherosclerosis. *J Biomech Eng*. 1993;115:588-94.
25. Friedman MH, Bargeron CB, Deters OJ, Hutchins GM, Mark FF. Correlation between wall shear and intimal thickness at a coronary artery branch. *Atherosclerosis*. 1987;68:27-33.
26. Ku DN, Giddens DP. Pulsatile flow in a model carotid bifurcation. *Arteriosclerosis* 1983;3:31-9.
27. Lipowsky HH. Microvascular rheology and hemodynamics. Microcirculation 2005;12:5-15.
28. Tsai AG, Cabrales P, Intaglietta M. Oxygen-carrying blood substitutes: a microvascular perspective. *Expert Opin Biol Ther*. 2004;4:1147-57.
29. Belhassen L, Pelle G, Sediame S, bachir D, Carville C, Bucherer C, Lacombe C, Galacteros F, Adnot S. Endothelial dysfunction in patients with sickle cell disease is related to selective impairment of shear stress-mediated vasodilation. *Blood* 2001;97:1584-9.
30. Lee AJ, Mowbray PI, Lowe GD, Rumley A, Fowkes FG, Allan PL. Blood viscosity and elevated carotid intima-media thickness in men and women: the Edinburgh Artery Study. *Circulation* 1998;97:1467-73.
31. Di Perri T, Guerrini M, Pasini FL, Acciabatti A, Pieragalli D, galigani C, Capecchi PL, Orrico A, Franchi M, Blardi P. Hemorheological factors in the pathophysiology of acute and chronic cerebrovascular disease. *Cephalalgia* 1985;5 Suppl 2:71-7.
32. Forconi S, Guerrini M, Agnusdei D, Pasini FL, di Perri T. Letter: Abnormal blood viscosity in Raynaud's phenomenon. *Lancet* 1976;2:586.
33. Carallo C, Pujia A, Irace C, De Franceschi MS, Motti C, Gnasso A. Whole blood viscosity and haematocrit are associated with internal carotid atherosclerosis in men. *Coron Artery Dis* 1998;9:113-17.
34. Forconi S, Guerrini M, Pieragalli D, Acciabatti A, Del Bigo C, Galigani C, Di Perri T. [Hemorrheological changes in ischemic heart disease]. *Ric Clin Lab* 1983;13 Suppl 3:195-208.
35. Caimi G, Hoffmann E, Montana M, Canino B, Dispensa F, Catania A, Lo Presti R. Haemorheological pattern in young adults with acute myocardial infarction. *Clin Hemorheol Microcirc* 2003;29:11-8.
36. Forconi S, Pieragalli D, Guerrini M, galigani C, Cappelli R. Primary and secondary blood hyperviscosity syndromes, and syndromes associated with blood hyperviscosity. *Drugs* 1987;33 Suppl 2:19-26.
37. Gori T, Lisi M, Forconi S. Ischemia and reperfusion: the endothelial perspective. *Clin Hemorheol Microcirc* 2006;35:31-4.
38. Nobili L, Schiavi G, Bozano E, de Carli F, Ferrillo F, Nonili F. Morning increase of whole blood viscosity in obstructive sleep apnea syndrome. *Clin Hemorheol Microcirc* 2000;22:21-7.
39. Antonova N, Velcheva I. Hemorheological disturbances and characteristic parameters in patients with cerebrovascular disease. *Clin Hemorheol Microcirc* 1999;21:405-8.
40. Mares M, Bertolo C, Terribile V, Girolami A. Hemorheological study in patients with coronary artery disease. *Cardiology* 1991;78:111-6.
41. Tozzi-Ciancarelli MG, Di Massimo C, Mascioli A, Tozzi E, Gallo P, Fedele F, Dagianti A. Rheological features of erythrocytes in acute myocardial infarction. *Cardioscience* 1993;4:231-4.
42. Nemeth N, Lesznyak T, Szokoly M, Furka I, Miko I. Allopurinol prevents erythrocyte deformability impairing but not the hematological alterations after limb ischemia-reperfusion in rats. *J Invest Surg* 2006;19:47-56.
43. Baskurt OK, Temiz A, Meiselman HJ. Effect of superoxide anions on red blood cell rheologic properties. *Free Radic Biol Med* 1998;24:102-10.
44. Engler RL, Schmid-Schonbein GW, Pavelec RS. Leukocyte capillary plugging in myocardial ischemia and reperfusion in the dog. *Am J Pathol* 1983;111:98-111.
45. Dormandy J, Ernst E, Bennett D. Erythrocyte deformability in the pathophysiology of the microcirculation. *Ric Clin Lab* 1981;11 Suppl 1:35-8.