Endothelial Function in Health and Disease

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12.1 Introduction

> It has been said that one is as old as one's arteries. In view of the supreme importance of endothelium in arterial function I would like to modify, or rather simplify, this statement by saying the one is as old as one's endothelium. [1]

Dr. Rudolf Altschul prefaced his book 'Endothelium: Its Development, Morphology, Function and Pathology' with this statement. Embedded in our current knowledge, this statement seems like an open door. However, it was made in 1954, a time during which relatively little was known on the subject. This contrasts with contemporary interest in the endothelium, supported by the publication of approximately 10,000 publications by 2014.

Since the discovery of the endothelium by Friedrich von Recklinghausen in mid-1800, appreciation of the morphology and functions of the endothelium saw slow progress. Notable milestones include the definition of Starling's 'Law of capillary exchange' which established the endothelium as a selectively permeable barrier [2] and the use of electron microscopy which enabled assessment of the endothelium's ultrastructure. Only recently has it been found that the endothelium produces

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vasoactive substances, such as prostacyclin in 1976 [3] and followed by the Nobel Prize winning work from Robert Furchgott who discovered the endothelium's ability to produce nitric oxide in 1980 [4].

We now know that the deceptively simple appearance of this single layer of cells belies the fact that it is 'the largest endocrine gland in the body' [5] with myriad of complex functions that play an integral part in vascular homeostasis. This chapter will focus on the (patho)physiological role of the vascular endothelium.

12.2 Physiological Functions of the Endothelium

The endothelium forms a barrier between blood-borne cells and macro-molecules and the underlying artery wall. Its permeability and integrity is regulated by transcellular (through endothelial cells) and paracellular (between endothelial cells) passage mechanisms. Transcellular transport takes place via transport vesicles (e.g. caveolae and vesiculo-vacuolar organelles) and is the primary means by which albumin, lipids, steroid hormones and fat-soluble vitamins cross the endothelium. Transport vesicles can also fuse into channels that traverse single cells, allowing the passage of leukocytes and solutes [6]. Furthermore, specialised fenestrae are present that control transcellular permeability to water and solutes. Paracellular transport occurs through the coordinated opening and closure of endothelial cell-cell junctions. Endothelial cell junctions also regulate contact-induced inhibition of cell growth, apoptosis, gene expression and new vessel formation [7]. The importance of controlling permeability is highlighted by the observation that increases in the permeability of the endothelium, which is linked to endothelium dysfunction and aids in the formation and progression of atherosclerosis [8].

In addition to the 'barrier function', the endothelium controls maintenance of vascular homeostasis. Through the autocrine secretion of substances, it exerts bidirectional control to form a finely balanced interdependent system [9] (Fig. 12.1). The most important functions of the endothelium include (1) regulation of vascular tone, (2) control of thrombosis and haemostasis, (3) immune and inflammatory responses and (4) facilitation of vascular growth, repair and remodelling. These functions of the endothelium are discussed below.

12.2.1 Regulation of Vascular Tone

12.2.1.1 Nitric Oxide

The endothelium regulates vascular tone through the rapid synthesis of vasodilators and vasoconstrictors (Fig. 12.1). Nitric oxide (NO) is an important and powerful vasodilator which is produced by the endothelium as a soluble gas with a short half-life (6–30 s in the artery wall and a few seconds in the blood). Its production involves a two-step oxidation of L-arginine to L-citrulline, with concomitant production of NO. This reaction is catalysed by NO-synthases (NOS) with the aid of cofactors, including tetrahydrobiopterin and nicotinamide adenine dinucleotide phosphate

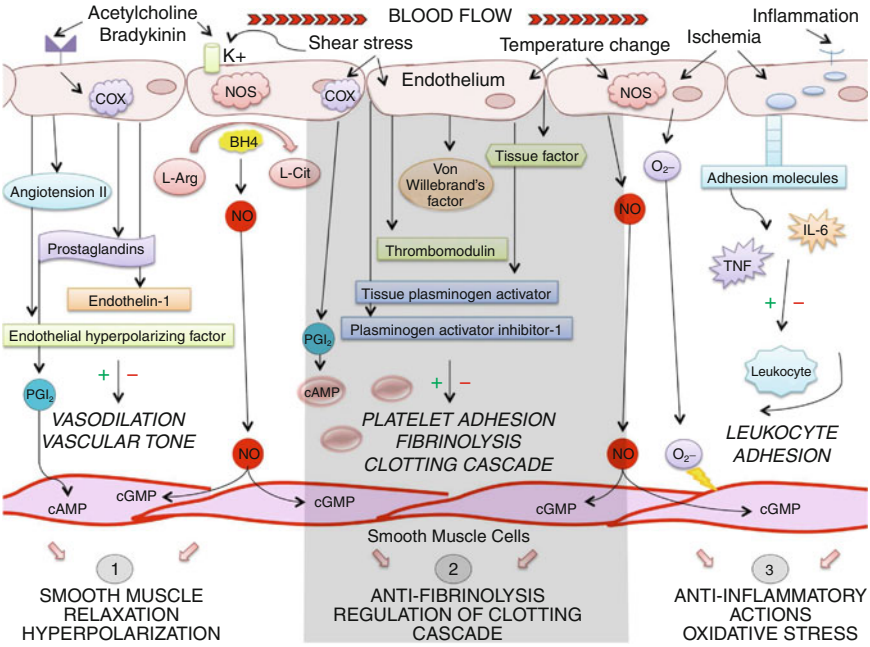

Fig. 12.1 The endothelium is responsible for a number of physiological functions, including (1) regulation of vascular tone, (2) control of blood fluidity and coagulation, and (3) regulation of inflammatory processes. *cAMP* cyclic adenosine monophosphate, *cGMP* cyclic guanosine monophosphate, *COX* cyclooxygenase, *BH4* tetrahydrobiopterin, *IL* interleukin, *TNF* tumour necrosis factor, l-*arg* L-arginine, l-*cit* L-citrulline, *NO* nitric oxide, *NOS* nitric oxide synthase, *O2*−superoxide (With permission from Marti et al. [9])

(NADPH) (Fig. 12.1). The NOS family plays a central role in the production of NO and consists of three different isoforms named after the tissues in which they were first identified: the neuronal (nNOS), inducible (iNOS) and endothelial (eNOS) isoforms. Many tissues can express more than one isoform, and all three may be constitutive or inducible [10]. Nonetheless, eNOS is the predominant form in endothelial cells and is the main source of endothelium-derived NO. eNOS is constitutively expressed and continuously produces small amounts of NO. eNOS can be stimulated by various hormones as well as haemodynamic factors. These stimuli induce an increase in intercellular Ca^{2+} that displaces the inhibitor caveolin from calmodulin to activate eNOS.

After production, NO diffuses to vascular smooth muscle cells (VSMCs) and activates guanylate cyclase, causing an increase in intracellular cyclic guanosine monophosphate (cGMP). This action leads to relaxation of the VSMCs and subsequent vasodilation. The importance of NO is supported by experimental work where inactivation of eNOS results in vasoconstriction and elevation in arterial blood pressure [11]. NO also exerts inhibitory effects on platelet aggregation, leukocyte adhesion and VSMC migration and proliferation, highlighting the importance of this hormone for vascular homeostasis [10].

12.2.1.2 Prostacyclin

The endothelium produces a family of prostaglandins through the catabolism of arachidonic acid by cyclooxygenases in response to mechanical and humoral stimuli. Prostacyclin (PGI_2) is a major member of this family and acts as a paracrine-signalling molecule and activates the prostacyclin receptors on VSMC and platelets. This stimulates adenylate cyclase and with a consequent increase in intracellular levels of cyclic adenosine monophosphate (cAMP), ultimately resulting in VSMC relaxation and inhibition of platelet activation [12]. The action of PGI_2 is closely related to that of NO since PGI_2 potentiates NO release (and vice versa). Nonetheless, PGI_2 seems less important for vascular control than NO, but plays a central role in the coagulation pathway (see Sect. 12.2.2).

12.2.1.3 Endothelium-Derived Hyperpolarizing Factor

Some of the endothelium-dependent vasodilation has generally been associated with hyperpolarization of the VSMCs and referred to a non-characterised factor called endothelium-dependent hyperpolarizing factor (EDHF) [13]. A number of candidate EDHFs have been suggested, including arachidonic acid metabolites, gaseous mediators (e.g. NO, hydrogen sulfide and carbon monoxide), reactive oxygen species, vasoactive peptides, potassium ions and adenosine. Irrespective of its exact nature, EDHF plays an important role in regulating vascular tone.

12.2.1.4 Endothelin

Endothelins are a family of potent vasoconstrictor peptides. Endothelin-1 (ET-1) is the predominant isoform and is primarily secreted by the endothelium in response to a variety of humoral and physical stimuli. After the production of ET-1 by the endothelium, it binds to the ET_A and ET_B receptors on VSMCs resulting in a sustained vasoconstriction. Endothelial cells also express ET_B receptors whose stimulation results in the release of NO and PGI2, leading to vasodilation (and therefore serves as a feedback mechanism to partially oppose the vasoconstrictive effects of VSMC-located $ET_{A/B}$-receptors). ET-1 also induces VSMC proliferation and growth in a dose-dependent manner, suggesting an important role for ET-1 to contribute to the atherosclerotic process [14].

12.2.1.5 Angiotensin

After cleavage of angiotensinogen to angiotensin (Ang) I via renin, this peptide is cleaved by the angiotensin-converting enzyme (produced by pulmonary and systemic vascular endothelium) into Ang II. The smooth muscle cell-localised AT1 receptor subtype mediates the predominant action of Ang II: vasoconstriction. These vasoactive actions are partly counteracted by the AT2 receptor, which causes vasodilatation [15]. Besides the vasoactive effects, Ang II leads to proliferation and growth of the VSMCs through activation of the AT1 receptor. Similarly to ET-1, the vasoconstrictive effects of AT1 are, at least partly, counterbalanced by a negative feedback loop in the vascular wall.

12.2.1.6 Thromboxane A_2

Thromboxane A_2 (TXA_2) is an end product of arachidonic acid metabolism and is produced by TXA_2 synthase. TXA_2 is primarily produced by platelets, but also by the endothelium. The primary physiological role of TXA_2 is platelet aggregation, but it has also been demonstrated to contribute to vasoconstriction [16].

12.2.1.7 Prostaglandin H_2

In contrast to most members of the prostaglandin family, prostaglandin H_2 (PGH_2) is a vasoconstrictor substance. PGH_2 is closely related to TXA_2 as both are formed during arachidonic acid metabolism. Furthermore, PGH_2 is the precursor of TXA_2 and exerts its vascular effects through the same receptors [17].

12.2.2 Control of Blood Fluidity and Coagulation

The endothelium actively maintains an anticoagulant and antithrombotic surface through several mechanisms (Fig. 12.1). First, the endothelium keeps circulating platelets in a quiescent state, mainly through release of NO and PGI_2 which synergistically increase cAMP content in platelets to repress activation and aggregation. Endothelial expression of ectonucleotidases also contributes to this process by converting ADP (a powerful trigger of platelet activation) to AMP and then adenosine. If platelet aggregation occurs, the release of serotonin and ADP from aggregating platelets will stimulate NO and PGI2 production to inhibit platelet aggregation and limit thrombus formation. Furthermore, vasodilation in response to NO and PGI_2 serve to mechanically impede the progression of the coagulation process [18].

Secondly, endothelial cells promote the activity of anticoagulant pathways (Fig. 12.2). Anticoagulation is achieved through expression of thrombomodulin which interacts with thrombin, forming a complex that prevents activation of platelets or the conversion of fibrinogen to fibrin, a key step in the coagulation cascade. This complex also activates protein C, which works in combination with protein S to inactivate two essential cofactors for blood coagulation, VIIIa and Va. The endothelial surface layer (glycocalyx) contains heparan sulfate proteoglycans which binds and activates antithrombin III to inactivate thrombin and factors IXa, Xa and XIa. Finally, endothelial cells regulate initiation of coagulation by inhibiting the activation of factor X [19]. In addition to the strong anticoagulation effects, the endothelium can also contribute to coagulation. Endothelial expression of tissue factor (TF) enhances the activity of factor VII, which ultimately activates thrombin to facilitate activation of platelets and release of the von Willebrand factor (vWF) to further promote platelet aggregation.

A third step in the coagulation pathway is the ability of the endothelium to influence fibrinolysis (Fig. 12.2) by the production of tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (uPA). These factors activate the liver-derived plasminogen into plasmin which then degrades fibrin. It is important to note that this activity is inhibited through the (endothelial) production of plasminogen activator inhibitor (PAI)-1 [19].

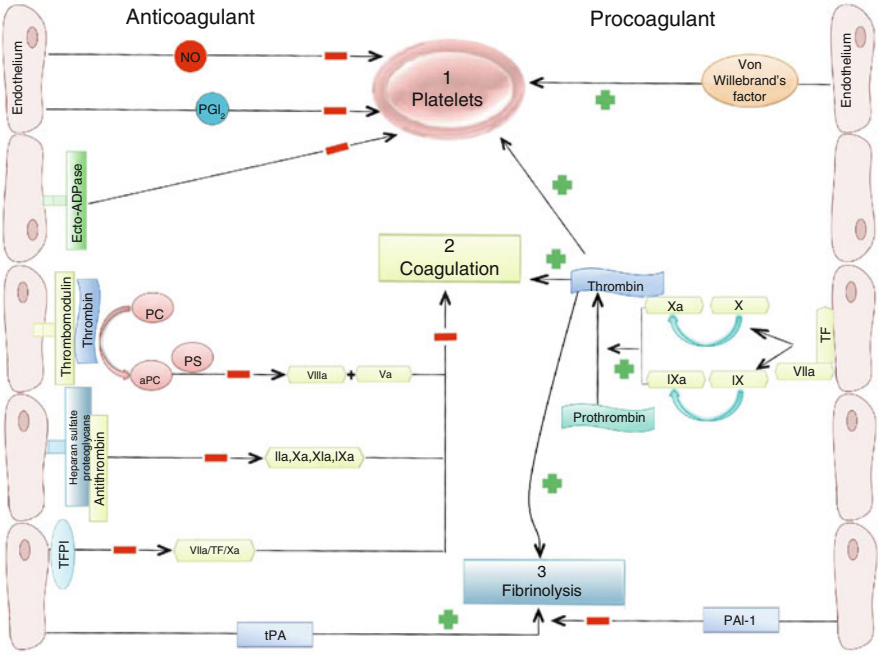

Fig. 12.2 Anticoagulant and procoagulant properties of the endothelium. The endothelium maintains blood fluidity through a balance between factors that either inhibit (*left*) or promote (*right*) (*1*) platelet activation, aggregation, and (*2*) coagulation, and inhibit (*left*) or promote (*right*) (*3*) fibrinolysis. *NO* nitric oxide, *PGI2* prostacyclin, *PC* protein C, *PS* protein S, *TF* tissue factor, *TFPI* tissue factor pathway inhibitor, *tPA* tissue-type plasminogen activator, *PAI-1* plasminogen activator inhibitor 1

12.2.3 Inflammatory Responses

Under noninflammatory conditions, interaction of endothelial cells with leukocytes is suppressed by inhibiting the endothelium-dependent production of adhesion molecules (Fig. 12.1). Also NO production inhibits the fusion of Weibel-Palade bodies with the surface of the endothelial cell and leukocyte activation. However, during inflammation, a rapid response to infectious microbes or injured tissues occurs, involving local recruitment and activation of leukocytes. The purpose of the inflammatory response is to kill microbes and remove cellular debris. Endothelial cell activation in response to inflammation can be divided into rapid responses (type I) and slower responses (type II).

Type I responses are rapid (<10–20 min), transient and independent of protein synthesis. These responses generally initiate a signalling cascade that increases intracellular Ca^{2+} levels to serve a number of purposes. First, this response facilitates increased NO and PGI_2 production, contributing to an increased blood flow and delivery of leukocytes. Secondly, increased Ca^{2+} levels enhance survival and migration of invading leukocytes and cause contraction of endothelial cells, which

opens gaps between adjacent endothelial cells and increase permeability for leukocytes. Finally, expression of P-selectin and platelet-activating factor (PAF) is initiated which promotes the binding and activation of leukocytes [20].

Type II activation of endothelial cells involves a more persistent form of activation. During sustained inflammation, leukocytes produce inflammatory cytokines (e.g. tumour necrosis factor-α (TNF-α) and interleukin-1 (IL-1)) which results in the increased transcription of genes responsible for expression of a pro-adhesive and prothrombotic endothelial cell phenotype (IL-8 and adhesion molecules). Increased expression of these factors contributes to further leukocyte migration, adhesion and extravasation into the inflamed tissue. Inflammatory cytokines also induce leakage of plasma proteins into the affected tissue. Since these responses require transcription and translation of new proteins, type II activation is slower in onset but has more sustained effects than type I activation (i.e. hours–days). Accordingly, endothelial cells contribute to restoration of normal tissue architecture or form a connective tissue scar in response to inflammation [21].

12.2.4 Facilitation of Remodelling, Growth and Repair

Remodelling or adaptation of the vasculature refers to a basic compensatory response intended to maintain the functional integrity of the vessel in the presence of (potentially harmful) haemodynamic, metabolic and inflammatory stimuli. Sustained exposure to such stimuli, especially in conjunction with CVD risk factors, eventually transforms the initial (protective) response into a self-perpetuating and pathogenic process that contributes to the development of atherosclerosis (Fig. 12.3) [22].

12.2.4.1 Remodelling

Straight portions of arteries are associated with laminar shear, which produces an atheroprotective genotype that mitigates the effects of risk factors. However, at flow dividers, disturbed flow impedes such atheroprotective functions and initiates increased expression of proatherogenic genes/proteins and (chronic) inflammatory responses. As a lesion forms and grows, matrix remodelling takes place, paving the way for abluminal expansion or outward growth of the growing atheroma that preserves the lumen of the artery and maintains blood flow. Ultimately, plaque growth can outstrip this compensatory enlargement of the artery wall, allowing the atheroma to encroach on the lumen and cause stenosis (Fig. 12.3). Smaller arterioles resist the atherosclerotic lesion formation. However, due to increases in pressure, these smaller vessels develop medial hypertrophy and intimal thickening, which sustains and aggravates hypertension [22].

Endothelial cells play a pivotal role in the process of remodelling. For example, early animal studies found that the presence of the endothelium is essential for arteries to adapt in luminal size [23]. When the endothelium is removed from an artery, exposure to elevations in shear stress does not induce a change in diameter. The adaptive response is therefore dependent on the endothelium, and more specifically,

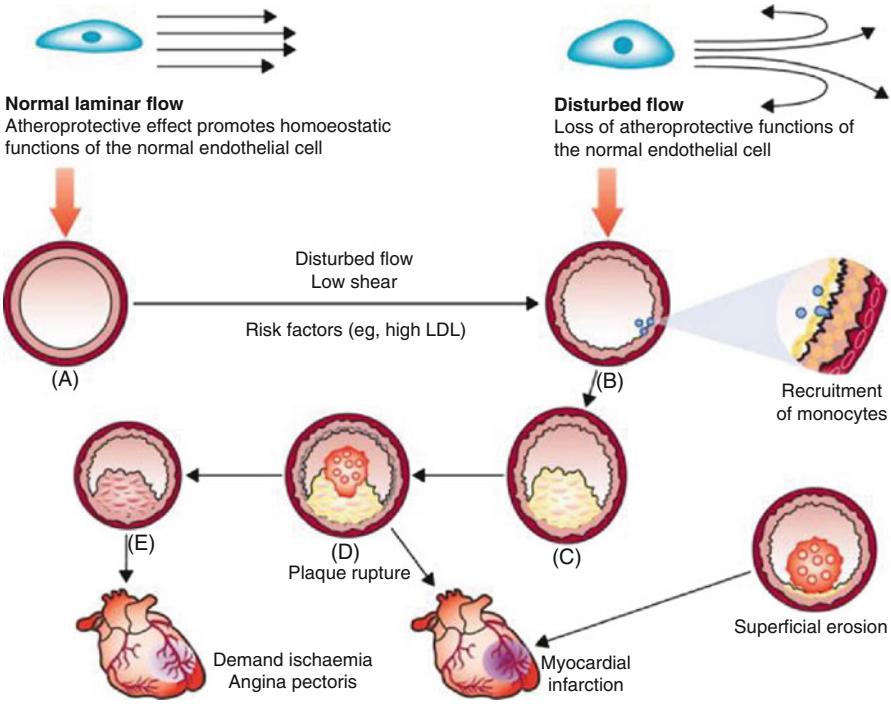

Fig. 12.3 Arterial remodelling influences the clinical consequences of atherosclerosis. Normal laminar shear stress maintains normal arterial calibre and properties (*A*). Disturbed flow characterised by a non-laminar, oscillatory flow promotes the upregulation of proatherogenic genes and recruitment of monocytes, as depicted to the right in the enlarged nascent plaque (*B*). Monocyte/macrophage accumulation yields a thin-capped, lipid-rich inflamed plaque (*C*), which can rupture and cause a thrombus (*D*), leading to myocardial infarction (*central bottom*). Alternatively, the plaque in (*E*) can undergo constrictive remodelling to promote flow-limiting stenosis that can cause demand ischaemia and angina pectoris. Less commonly, superficial erosion (*bottom right*) can cause myocardial infarction (With permission from Heusch et al. [22])

dependent on gene expression of eNOS and suppression of the bioavailability of ET-1 [24]. Also, immediate changes in vessel diameter in response to elevations in shear stress are dependent on an intact endothelium that releases vasoactive substances [25], highlighting the importance of the endothelium to respond (acutely and chronically) to changes in shear.

12.2.4.2 Repair

During the past decade, evidence supported the presence of repair mechanisms of damaged or 'old' endothelial cells by bone marrow-derived cells: endothelial progenitor cells (EPCs). These immature cells have the capacity to maintain endothelial integrity and function by differentiating into mature endothelial cells, replacing damaged endothelial cells and initiating neovascularisation [26]. Vascular injury mobilises circulating EPCs, which localise at the site of damage where they divide,

proliferate and adhere to the sub-endothelium promoting growth of new endothelium. Endothelial injury causes a cytokine-mediated release of stromal-derived factor-1 (SDF-1) which, in turn, determines recruitment and proliferation of EPCs. Intact NO bioavailability is linked to efficient mobilisation and functionality of EPCs, which may explain why the number of CVD risk factors is inversely related to the number and migratory activity of circulating EPCs. Impairment in the ability and/or efficacy of repair mechanisms of the endothelium logically follows the presence of CVD risk factors and progression of atherosclerosis [27].

12.3 Haemodynamic Forces Affecting the Endothelium

12.3.1 Shear Stress

Blood imparts a tangential frictional force on the endothelial surface, typically referred to as shear stress (SS). Endothelial cells' ability to detect SS and other haemodynamic forces is mediated by different sensing mechanisms, including ion channels, caveolae, G-protein-coupled receptors, tyrosine kinase receptors, cell adhesion molecules, glycocalyx, primary cilia and the cell cytoskeleton and the lipid bilayer of the cell membrane [28]. Mechanoactivation of the signalling pathways results in the activation of transcription factors, leading to modulation of gene expressions in endothelial cells. Importantly, responses to SS are not simply linked to the absolute level of SS, but also the type of SS (i.e. laminar or oscillatory).

Straight portions of arteries are exposed to a steady, laminar SS. Sustained elevation of such types of SS generates an atheroprotective phenotype. This is highlighted by the expression of approximately 3,000 cultured endothelial cell genes after exposure to elevations in laminar SS [29]. Gene expressions that are upregulated by laminar SS involve growth factors (e.g. FGF, TGF-β), vasodilators (e.g. NO, PGI_2), antithrombotic components (e.g. TPA, thrombomodulin, COX-2) and endogenous antioxidants, whilst downregulation is observed in adhesion molecules (e.g. VCAM-1), vasoconstrictors (e.g. ET-1) and coagulation factors (e.g. PAI-1) [28]. Conversely, regions of disturbed blood SS (e.g. branch points, curvatures, poststenotic regions), typically include periods of reciprocating flow reversal that create oscillating wall SS. Experiments suggest that oscillating SS patterns produce a proatherogenic endothelial cell phenotype, characterised with decreased eNOS mRNA and increased VCAM-1, ICAM-1, MCP-1, ET-1 and NADPH oxidase 4 [28, 30].

The impact of elevation in SS is well established from animal and human experiments. Subjects exposed to 8-week repeated exposure to elevated SS via handgrip training demonstrate improvement in vascular function and structure, whilst such adaptations were not present when the exercise-induced elevation in SS was artificially attenuated [31]. Furthermore, the detrimental effect of oscillatory SS is confirmed in humans in vivo as this leads to a dose-dependent decline in endothelial function [32]. These observations highlight the importance of the magnitude *and* pattern of SS for endothelial function and vascular structure.

12.3.2 Transmural Pressure and Cyclic Strain

Elevations in blood pressure exposes endothelial cells to an increased transmural force and increased cyclic strain, both of which are signals that can alter endothelial cell phenotype. High transmural pressure directly affects endothelial cell NO production [33]. Both in vitro and in vivo experiments in animals and humans demonstrate that exposure of arteries to short-term increases in transmural pressure depresses endothelium-dependent vasodilation by activating ROS-dependent mechanisms. More specifically, elevation in transmural pressure increases intracellular Ca^{2+} concentrations and protein kinase C activation, which ultimately results in NADPH oxidase activation and causes an increase in ROS production [34]. The increased oxidative stress contributes to a proatherogenic phenotype of endothelial cells and, consequently, contributes to the atherosclerotic process [34].

Elevation in pressure across the cardiac cycle also produces an increase in the rhythmic stretching (cyclic strain) of the vessel. Data obtained from in vitro cell culture preparations suggest that cyclic strain produced an antiatherogenic endothelial cell phenotype [35]. However, more recent data obtained from an isolated vessel preparation suggest a proatherogenic phenotype and increased ROS production [36]. Although speculative, the discrepancies in results between whole vessel preparations versus endothelial and smooth muscle cell culture may reflect the importance of crosstalk between endothelial and VSMCs. Research using co-cultured endothelial and VSMCs is required to determine the ultimate importance of cyclic strain on the phenotype of the endothelium.

12.4 CVD Risk Factors Affecting the Endothelium

Loss of the delicate balance of the various functions of the endothelium leads to a dysfunctional state in which the vasoconstricting, prothrombotic and proliferative characteristics of the endothelium predominate, ultimately facilitating the process of atherosclerosis. Below, we describe how important cardiovascular risk factors influence this balance.

12.4.1 Dyslipidaemia

Dyslipidaemia relates to excessive levels of low-density lipoproteins (LDL) and/or low levels of high-density lipoproteins (HDL). High levels of LDL, and especially oxidised LDL, inhibit eNOS activity through inactivation of eNOS and ‘eNOS uncoupling’. This latter process is initiated by superoxide, which reacts with NO to form peroxynitrite. Peroxynitrite then oxidises the eNOS-cofactor tetrahydrobiopterin, which causes the generation of superoxide [37]. This process reduces NO production and potentiates the pre-existing oxidative stress. Conversely, high levels

of HDL protect the endothelium through the prevention of LDL-induced eNOS uncoupling and upregulation of eNOS mRNA and protein levels, anti-inflammatory effects through inhibition of NF-κB, as well as antithrombotic activity [38].

12.4.2 Diabetes

Hyperglycaemia, a common feature in diabetes type 1 and 2, induces reduced NO bioavailability as a result of oxidative stress and eNOS uncoupling. Hyperglycaemia increases synthesis of vasoconstrictor prostanoids (e.g. PGH2, TXA) [39], causing an immediate decline in endothelial function. Another pathway by which diabetes impacts the endothelium is production of insulin, which normally acts as a vasodilator and stimulates endothelial NO production to facilitate glucose uptake in the muscle. In diabetes, this signalling pathway is inhibited, possibly via excess production of free fatty acids and inflammatory cytokines from adipose tissue [39] (especially in type 2 diabetes).

12.4.3 Hypertension

In addition to the effects of elevation in transmural pressure (Sect. 12.3.2), Ang II plays a major role in hypertension through profound vasoconstriction, increased renal sodium absorption and elevated pressor responses. This high blood pressure stimulates oxidative stress by enhancing NADPH oxidase activity, increasing media stress and stimulation of mechanoreceptors. Ang II also influences remodelling of the vessel wall by stimulating Ca^{2+} release leading to vasoconstriction that may become embedded as deposition of extracellular matrix occurs. In addition, Ang II enhances all stages of the inflammatory response [40]. These effects of Ang II establish a vicious cycle where hypertension begets hypertension through its adverse effects on the endothelium and vascular structure.

12.4.4 Obesity

Obesity is independently associated with endothelial dysfunction [41]. Visceral adipose tissue acts as an endocrine organ and is capable of producing proinflammatory adipokines (e.g. leptin, resistin and adiponectin). Leptin induces oxidative stress in endothelial cells and stimulates the secretion of the proinflammatory cytokines TNF-α and IL-6 causing a state of chronic low-grade inflammation. Resistin inhibits glucose uptake in skeletal muscle cells and stimulates ET-1 production. Conversely, obesity is typically characterised by a reduced production and activity of adiponectin [42]. Adiponectin has antiatherogenic properties and stimulates insulin sensitivity, reduces the expression of adhesion molecules, inhibits the transformation of macrophages into foam cells and reduces VSMC proliferation. Furthermore, obesity is associated with increased plasma levels of free fatty acids which may contribute to endothelial dysfunction [41].

12.5 Concluding Remarks

The endothelium is the central regulator of vascular homeostasis. Changes in endothelial cell phenotype support vessel repair, remodelling and resolution of infection or inflammation. Whilst these alterations are usually transient, prolonged exposure to harmful stimuli activates various processes that ultimately lead to endothelial dysfunction. This process, characterised by reduced NO bioavailability, represents a critical step in the atherosclerosis process and is present long before overt pathophysiological changes occur. Therefore, the endothelium remains an attractive target for (1) early prediction of future CVD and (2) therapies preventing CVD.

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