

## Effect of drug-eluting stents on endothelial dysfunction

Drug-eluting stents are considered a breakthrough in the treatment of coronary artery disease owing to their ability to reduce rates of restenosis and the subsequent need for target lesion revascularization. The literature has limited reports of long-term results of drug-eluting stent implantation with respect to vascular integrity and coronary endothelial function. Thus, we review the currently available data with use of different drug-eluting stents and their effects on endothelial cell dysfunction.

**KEYWORDS:** drug-eluting stent ■ endothelial cell dysfunction ■ stent thrombosis ■ vasomotor reactivity

Widespread use of drug-eluting stents (DES) in clinical practice has had a tremendous impact in reducing the clinical events and angiographic restenosis after percutaneous coronary intervention [1–3]. However in early 2004, reports began to suggest an increased incidence of early, late and very late stent thrombosis (ST) after first-generation DES implantation, especially after discontinuation of dual antiplatelet therapy [4]. One of the primary mechanisms proposed for subacute thrombosis has been delayed or incomplete endothelialization of the vessel wall at the site of stent deployment because of the presence of the drug, the polymer, or both, leading to thrombus formation [5]. Thus, prolonged dual antiplatelet therapy has been recommended to reduce late ST, a rare but potentially life-threatening complication of DES use that has become an increasingly controversial issue in interventional cardiology [6–8]. In recent years, several studies have reported long-term endothelium-related vascular dysfunction in the nonstented segments of the coronary arteries following DES implantation [9–11]. Although the concept of DES-induced endothelial dysfunction leading to late ST is hypothetical, without studies showing causal association, previous studies have demonstrated that coronary endothelial vasodilator dysfunction is an independent predictor of atherosclerotic disease progression and cardiovascular event rates [12]. We review the currently available data with use of different DES types and their effects on endothelial dysfunction.

### Endothelial function & dysfunction

Endothelium is a single layer of cells that has a multitude of functions, including vasomotor regulation, maintaining the balance between

coagulation and fibrinolysis, being the mediator of inflammation by allowing leukodiapedthesis, and being one source promoting vasculogenesis (FIGURE 1) [13].

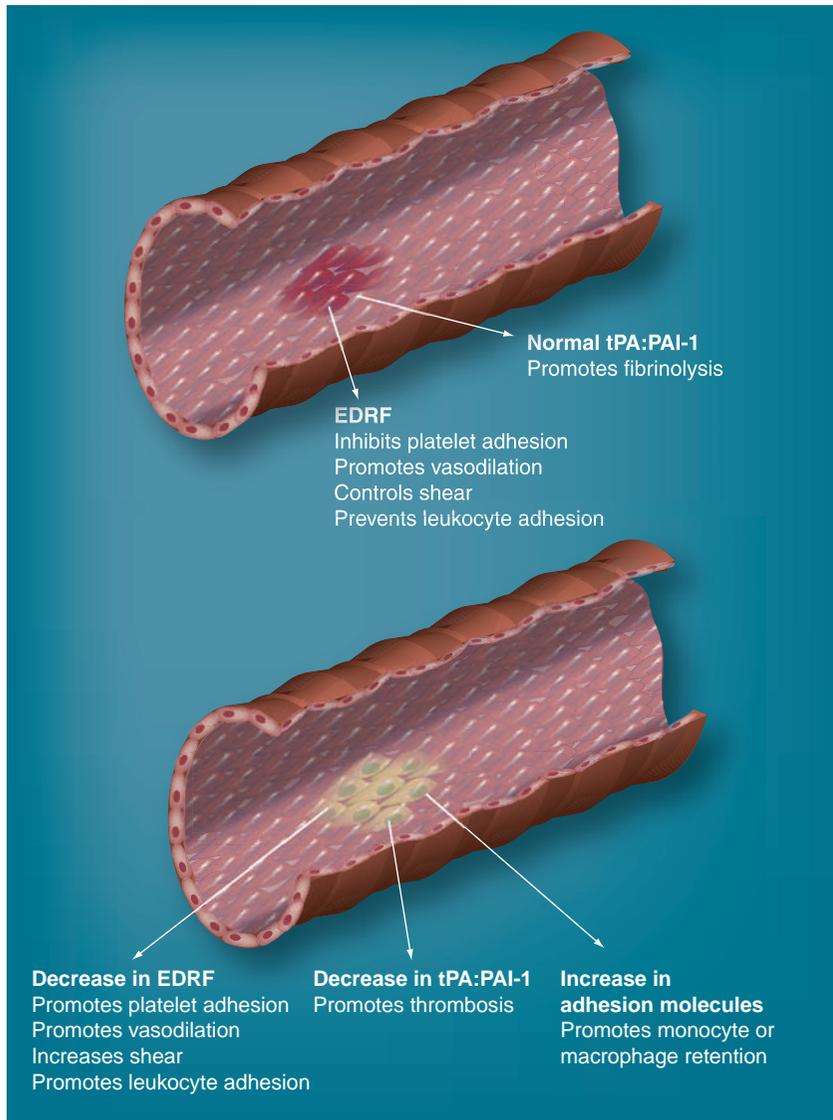
Nitric oxide (NO) is primarily secreted by the endothelial cells and plays a key role in coronary vasomotor function, inflammation, thrombosis, atherosclerosis and arterial neointima formation [14]. Endothelial dysfunction is commonly defined as a state of reduced NO availability. This can be attributed to decreased availability of L-arginine or impaired expression of endothelial NO synthase (eNOS), or increased metabolism of NO itself.

Clinically, endothelial function is most often assessed as a vasodilator response to pharmacological or mechanical stimuli [15,16]. Under basal conditions, there is no or minimal resting sympathetic tone in the heart vasculature and, thus, there is no effect of denervation on resting perfusion. During sympathetic activation such as exercise, coronary tone is modulated by norepinephrine released from myocardial sympathetic nerves, as well as by circulating norepinephrine and epinephrine [17]. In conduit arteries, sympathetic stimulation leads to  $\alpha_1$  constriction as well as  $\beta_2$ -mediated vasodilation. The net effect is to dilate the pericardial coronary arteries. This dilation is potentiated by concomitant flow-mediated vasodilatation from metabolic vasodilation of coronary resistance vessels. When NO-mediated vasodilation is impaired,  $\alpha_1$  constriction predominates and can dynamically increase stenosis severity in asymmetrical lesions where the vessel is compliant. This is one of the mechanisms that can provoke ischemia during sympathetic activation from cold pressor testing or exercise [12,17].

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**Figure 1. Endothelial function.** Endothelium is a complex endocrine and paracrine organ that affects vasoregulation, smooth muscle cell proliferation, platelet aggregation, monocyte and leukocyte adhesion, and thrombosis, all of which are cardinal features in the pathogenesis and progression of atherosclerosis. EDRF: Endothelium-derived relaxing factor; PAI: Plasminogen activator inhibitor; tPA: Tissue plasminogen activator. Adapted with permission from [13].

Beside incomplete endothelialization or dysfunctional endothelium, there are other proposed mechanisms leading to paradoxical vasoconstriction in diseased coronary arteries as normal coronary arteries dilate in response to exercise [12]. The paradoxical response of the stenotic arteries during exercise has been attributed to a variety of potential mechanisms including: reduced NO availability at the site of the stenosis (endothelial dysfunction); enhanced vasoconstrictor response due to an increase in circulating catecholamines (enhanced sympathetic stimulation); enhanced platelet aggregation due to turbulent blood flow through the stenotic lesion with release

of thromboxane A2 and serotonin; and flow-induced (passive) collapse of the normal vessel segment within the stenosis (Venturi effect).

Sympathetic and vagal nerves innervate coronary conduit arteries and segments of the resistance vasculature. Neural stimulation affects vascular tone through mechanisms that alter vascular smooth muscle cells as well as by stimulating the release of NO from the endothelium. Diametrically opposite effects can occur in the presence of risk factors that impair endothelium-dependent vasodilation. Resistance arteries dilate in response to acetylcholine (ACh), resulting in increased coronary flow [12]. In conduit arteries, ACh normally causes mild coronary vasodilation (FIGURE 2). This reflects the net action of a direct muscarinic constriction of vascular smooth muscle counterbalanced by an endothelium-dependent vasodilation caused by direct stimulation of NOS and an increased flow-mediated dilation from concomitant resistance vessel vasodilation. The response in humans with clinical or subclinical atherosclerosis is distinctly different. The resistance vessel dilation to ACh is attenuated and the reduction in flow-mediated NO production leads to net paradoxical epicardial conduit artery vasoconstriction, which is particularly prominent in stenotic segments (FIGURE 2) [12]. In the cardiac catheterization laboratory, endothelial function can be estimated by measuring the coronary vasoreactivity in response to titrating doses of intracoronary infusion of ACh.

In contrast to ACh, nitroglycerin (NTG) is an endothelium-independent vasodilator. It dilates epicardial conduit arteries and small coronary resistance arteries but does not increase coronary blood flow in the normal heart. Since it is an endothelium-independent vasodilator, it can induce coronary vasodilation even in the presence of atherosclerosis and associated risk factors [18].

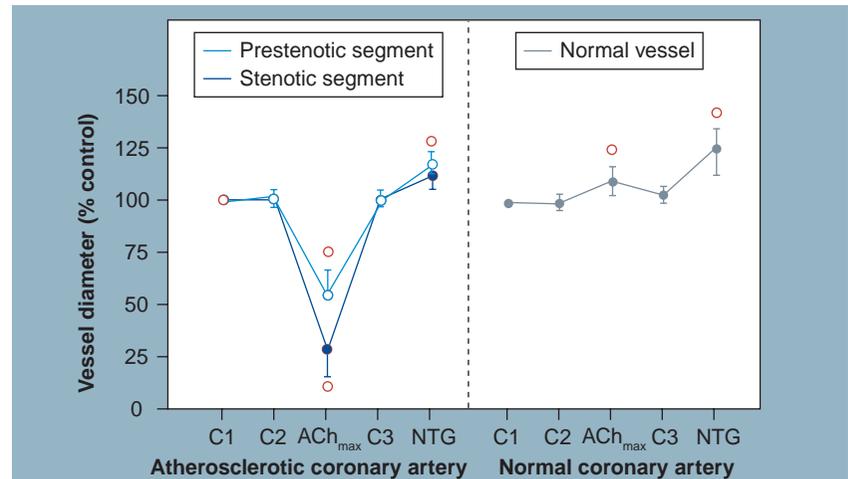
Coronary resistance arteries and arterioles also regulate their diameter in response to changes in local shear stress [19]. This is found to be endothelium-dependent and NO-mediated. Flow-mediated vasodilation leads to vasodilation in normal coronary arteries, but by contrast, it causes paradoxical vasoconstriction in coronary artery disease or in the presence of associated risk factors. Dynamic bicycle exercise or rapid atrial pacing has been used to induce flow-mediated coronary vasodilation to assess endothelial function in the cardiac catheterization laboratory [12].

Investigation of endothelial function was performed early in the balloon angioplasty era. As a response to healing after the barotrauma

of balloon angioplasty alone, the residual endothelial cells are dysfunctional and remain so for a variable period of time (FIGURE 3) [20,21]. However, the healing of an injured vessel segment after percutaneous transluminal balloon angioplasty is associated with re-endothelialization and improvement of endothelial function or even normalization of response at follow-up when assessed months later. It was also shown that successful angioplasty reverses the vasoconstrictive response of stenotic arteries during exercise [20]. The mechanism by which balloon angioplasty improves or restores coronary vasomotion is attributed mainly to an increase in endothelial cell number and function with enhanced NO release during exercise [21,22]. Clinical outcomes are associated with coronary vasoreactivity. Suwaidi *et al.* have reported that in a series of patients with nonobstructive coronary lesions stratified by endothelial dysfunction, those who had normal function had no subsequent cardiac events while those who had severe dysfunction had a 14% (six out of 42) cardiac event rate, including two deaths and one myocardial infarction (TABLE 1) [23].

Implantation of balloon expandable stents is associated with deep vessel wall injury as well as trauma to the coronary endothelium. This trauma sets in motion the inflammatory reaction and a healing response that eventually leads to re-endothelialization during follow-up. In the first month after the implantation of a bare-metal stent (BMS), a new immature endothelial layer covers the stent struts, re-establishing an endothelial lining of the coronary vessel wall. Abnormal and immature endothelial cells have a thrombogenic surface, promote adherence of various circulating monocytes and platelets, and facilitate platelet aggregation, leukocyte infiltration and vascular smooth muscle cell proliferation. Endothelial dysfunction is even more pronounced after BMS compared with balloon angioplasty [24,25]. BMS implantation abolishes paradoxical vasoconstriction of coronary stenosis in the stented segment and renders a previous vasoresponsive vessel to a rigid tube. Although initial vasomotor studies using intracoronary infusion of ACh suggested possible long-term impaired endothelial function in the proximal and distal adjacent segments after BMS implantation, Maier *et al.* showed maintenance of near-normal vasomotor response to exercise at the proximal and distal vessel segments (FIGURE 4) [26].

Several landmark studies using DES have demonstrated an impressive reduction in the rates of angiographic restenosis and associated clinical events [1,3]. However, long-term results of



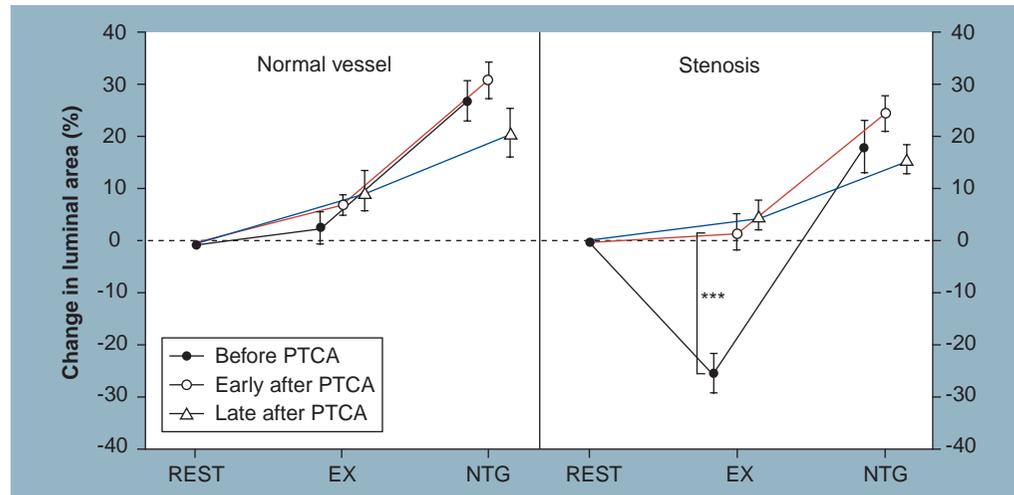
**Figure 2. Assessment of endothelial dysfunction using intracoronary acetylcholine injection in normal versus atherosclerotic coronary artery.** Intracoronary infusion of acetylcholine leads to vasodilation in normal coronary arteries. In the diseased coronary arteries, it leads to paradoxical vasoconstriction. ACh: Acetylcholine; NTG: Nitroglycerin. Adapted with permission from [15].

DES implantation with regard to vascular integrity and coronary endothelial function were not studied in detail until recently.

### First-generation drug-eluting stents

The sirolimus-eluting stent (SES; Cypher™, Cordis Corporation, FL, USA) is one of the first-generation DES. Sirolimus inhibits smooth muscle cells as well as endothelial cell proliferation via cell cycle arrest in the late G1 phase by forming a complex with FK506-binding protein (FKBP12) that inhibits the protein Ser-Thr kinase mammalian target of rapamycin (mTOR), with mTOR being a central element in signaling pathways involved in the control of cell growth and proliferation [27,28]. Recently, concerns have been raised that SES use could be associated with increased rates of ST owing to delayed or absent endothelialization [4,6]. Long *et al.* showed that acute *in vitro* sirolimus treatment as well as genetic deletion of the sirolimus-receptor isoform FKBP12.6 increased PKC-mediated eNOS threonine 495 phosphorylation, which could result in decreased vascular NO production and subsequent endothelial dysfunction [29]. Impairment of endothelial recovery may theoretically predispose the patient to poor long-term effects such as ST, progression of coronary lesions and negative vascular remodeling [5,7].

Togni *et al.* studied coronary vasomotor response to bicycle exercise after SES versus BMS in 24 patients with *de novo* lesions [11]. All the baseline characteristics and procedural data were comparable with regards to stented vessel, stent length and diameter between the two groups.



**Figure 3. Exercise-induced vasomotion following balloon angioplasty.** Balloon angioplasty has been found to improve exercise-induced coronary vasomotion after percutaneous coronary intervention. EX: Exercise; NTG: Nitroglycerin; PTCA: Percutaneous transluminal coronary angioplasty; REST: Resting. Adapted with permission from [22].

Stent implantations were performed according to standard guidelines. Vasoactive medications were discontinued at least 48 h before catheterization. Diagnostic catheterization was performed with standard techniques at baseline. At the end of diagnostic catheterization, biplane coronary angiography was performed with the patient's feet attached to the supine bicycle ergometer. Exercise began at 50 or 75 W and workload was increased every 2 min in increments of 25 or 50 W. The catheter was left in place during exercise. Coronary angiography was carried out at the end of each exercise level and at maximal exercise in deep inspiration. At the end of the exercise test, all patients received 1.6 mg NTG sublingually, and 5 min later coronary angiography was repeated. NTG was administered routinely to assess endothelium-independent vasodilatation. Both

groups were studied at 6 months ( $\pm$  1 month) after the intervention. Minimal luminal diameter, stent diameter, and proximal, distal and reference vessel diameter were determined using quantitative coronary angiography. Angiographic as well as intravascular ultrasound (IVUS) data indicated normal structural appearance of the target vessel after SES. These findings, however, could not exclude functional abnormalities of peri-stent and in-stent segments after SES implantation. This study revealed exercise-induced vasoconstriction of coronary artery segments adjacent to SES (i.e., within a range of 5–10 mm proximal and distal to the stent edges [persistent region]) (FIGURE 5). Outside this region (i.e., 10–20 mm proximal and distal to the stent edges) exercise-induced vasomotion was normal, comparable to patients with BMS, suggesting that sirolimus may induce

**Table 1. Long-term-follow-up of patients with mild coronary artery disease and endothelial dysfunction.**

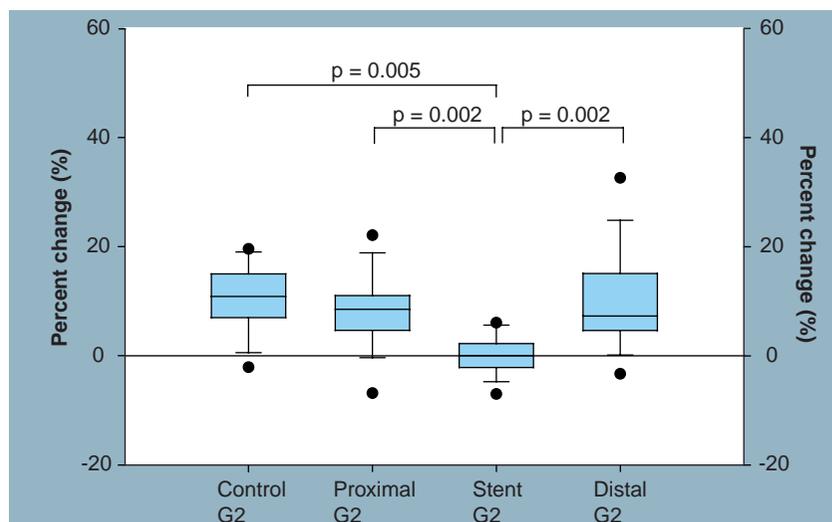
End point (157 patients)	Normal endothelial function (n = 83)	Mild endothelial dysfunction (n = 32)	Severe endothelial dysfunction (n = 42)
Cardiac death (%)	0	0	2 (4.8)
Myocardial infarction (%)	0	0	1 (2.4)
Heart failure (%)	0	0	2 (4.8)
CABG (%)	0	0	2 (4.8)
PCI (%)	0	0	3 (7.1)
Cardiac event (%)	0	0	6 (14.3)

*Patients with normal endothelial function had no subsequent events while patients with endothelial dysfunction had increased event rates directly proportional to the severity of the endothelial dysfunction.*  
 CABG: Coronary artery bypass graft; PCI: Percutaneous coronary intervention.  
 Data taken from [23].

vascular damage with endothelial dysfunction, thereby reducing NO availability in the persistent region. Paradoxical vasoconstriction in a vessel segment not subjected to percutaneous transluminal coronary angioplasty barotraumas may be due to diffusion of the antiproliferative drug from the stent to peri-stent region, inducing endothelial dysfunction. This hypothesis remains to be tested *in vivo*. Similarly, Hofma *et al.* demonstrated endothelial dysfunction in the persistent region after SES with ACh testing [10].

Cypher stents used in this study contained a 5- $\mu$ m-thick coating of the drug sirolimus mixed with nonerodable polymers, topped with a layer of drug-free polymer to allow the release of approximately 80% of the drug within 30 days after implantation [30]. Since most of the drug sirolimus is eluted from the polymer coating of the stent by 28 days [31] and is reportedly fully eluted by 60 days [32], abnormal vasomotion observed at 6 months in this study should not be the result of a direct effect of sirolimus itself. However, the induction of a persistent abnormality in intact or regenerating endothelium occurring while sirolimus was present cannot be excluded either. Alternatively, the polymer from which the drug elutes, which may have contributed to a case of a marked hypersensitivity reaction [32], could be involved in the abnormal vasomotion observed. In addition, the finding of paradoxical vasoconstriction could be the result of delayed endothelialization, with inadequate endothelial coverage leading to insufficient NO release to promote normal vasodilation with exercise. Vasomotion within the stented segment was abolished as expected. Sublingual NTG was associated with maximal vasodilatation of the proximal and distal vessel segments regardless of stent type.

Jabs *et al.* further studied the possible molecular mechanisms that mediate endothelial dysfunction induced by sirolimus [33]. To mimic the continuous sirolimus exposure of a stented vessel, they used Wistar rats that underwent drug infusion with an osmotic pump for 7 days. Both endothelium-dependent as well as endothelium-independent vasodilator responses were significantly impaired after 7 days of sirolimus treatment [33]. There was a 40% reduction in NO bioavailability. There was also increased transmural reactive oxygen species (ROS) production. This ROS increase was triggered by NADPH oxidase expression and membrane association as well as stimulation of mitochondrial ROS release. The systemic polymer containing the drug may also be the trigger of local inflammation. Coronary inflammation is responsible for delayed re-endothelialization of

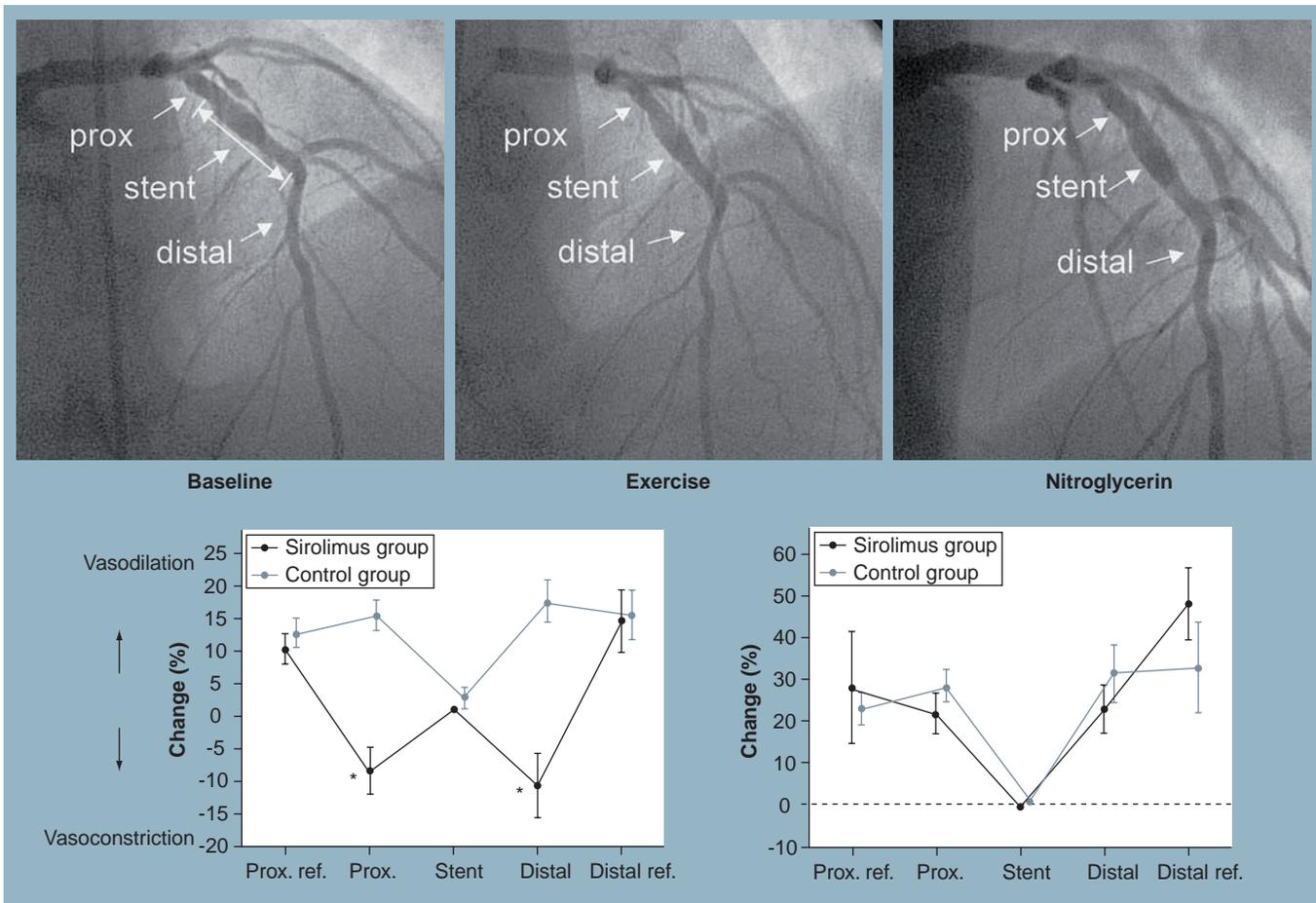


**Figure 4. Exercise-induced vasomotion following bare-metal stent implantation.** Bare-metal stent implantation abolishes paradoxical vasoconstriction of the coronary stenosis but does not adversely affect vasomotion of the adjacent vessel segments. Adapted with permission from [26].

the stent and vessel wall, which eventually results in a delayed vascular healing response. Sirolimus impairs normal healing processes of the injured arterial wall by inhibiting endothelial cell proliferation. In addition, sirolimus was demonstrated to enhance tissue factor expression in human endothelial cells [34], and inhibit the migration and proliferation of endothelium progenitor cells, which may play an important role in endothelium regeneration [26]. These findings suggest that SES may impair both the endothelial regeneration and the ability of endothelial cells to exert their normal function. Poor endothelialization means that stent struts are in direct contact with blood and its elements. Human autopsies showed insufficient endothelial coverage and delayed arterial healing with SES, regardless of the delay from implantation [35]. These findings are complemented by angiography [36] and optical coherence tomography (OCT) imaging [37], which showed incomplete endothelialization and uncovered stent struts 3–6 months after SES implantation. Complete or partial lack of re-endothelialization of the stent and vessel surface favoring platelet adhesion and aggregation may eventually cause thrombus formation.

These findings of incomplete endothelial coverage within the stented segment and dysfunctional endothelium proximal and distal to the stent suggest that in a subset of patients, SES may be vulnerable for late-ST and persistent restenosis.

Paclitaxel is an antimicrotubule agent that interferes with neointimal vascular smooth muscle cell accumulation and proliferation [38].



**Figure 5. Exercise-induced vasoconstriction of coronary artery segments adjacent to a sirolimus-eluting stent.** Compared with bare-metal stent, sirolimus-eluting stent implantation resulted in impairment of exercise-induced vasodilation in the adjacent segments of the coronary artery lesion. However, vasodilatory response to nitroglycerin is maintained. Distal Ref.: Distal reference; Prox.: Proximal segment; Prox. Ref: Proximal reference segment. Adapted with permission from [11].

Togni *et al.* also studied long-term effects (2–12 months) of paclitaxel-eluting stents (PES; Taxus™, Boston Scientific Corporation, MA, USA) on vascular function compared with its BMS counterparts [9]. Coronary vasomotion was evaluated by biplane quantitative coronary angiography at rest and during supine bicycle exercise according to the previous protocol in 27 patients with significant coronary artery disease. Reference vessels showed exercise-induced vasodilatation in both groups. Vasomotor function within the stented segment was again abolished as expected. In the BMS group, the adjacent segments proximal and distal to the stent showed exercise-induced vasodilatation. By contrast, there was exercise-induced vasoconstriction of the proximal and distal vessel segments adjacent to the PES (FIGURES 6 & 7). After sublingual NTG, both the proximal and distal segments dilated in both groups. In addition, exercise-induced vasoconstriction adjacent to PES correlated inversely with the time interval

after stent implantation; suggesting improvement in vascular function over time, indicating delayed vascular healing.

Finn *et al.* reported that sirolimus or paclitaxel released from a DES impaired the normal healing process of the injured arterial wall, even over a period of 40 months after implantation, and the heterogeneity of healing in the stents was associated with late-stent thrombi [35]. Kim *et al.* showed *in vivo* that both SES and PES can equally impair endothelial function and that their effects were demonstrable 6 months after implantation, especially in the arterial segments distal to the DES 6 months after stenting [39].

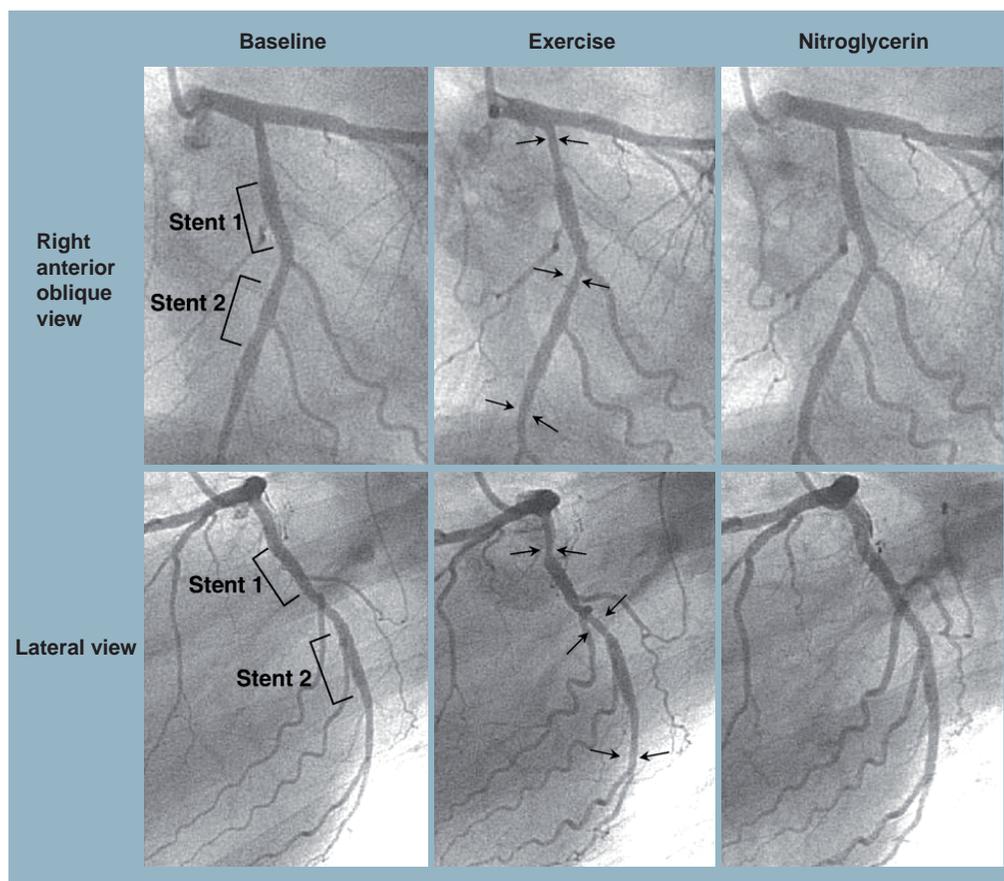
### Second-generation drug-eluting stents

Second-generation DES have been developed in order to improve the biologic healing process. These stents employ newer formulations of biodegradable polymers and drugs specifically developed for local applications.

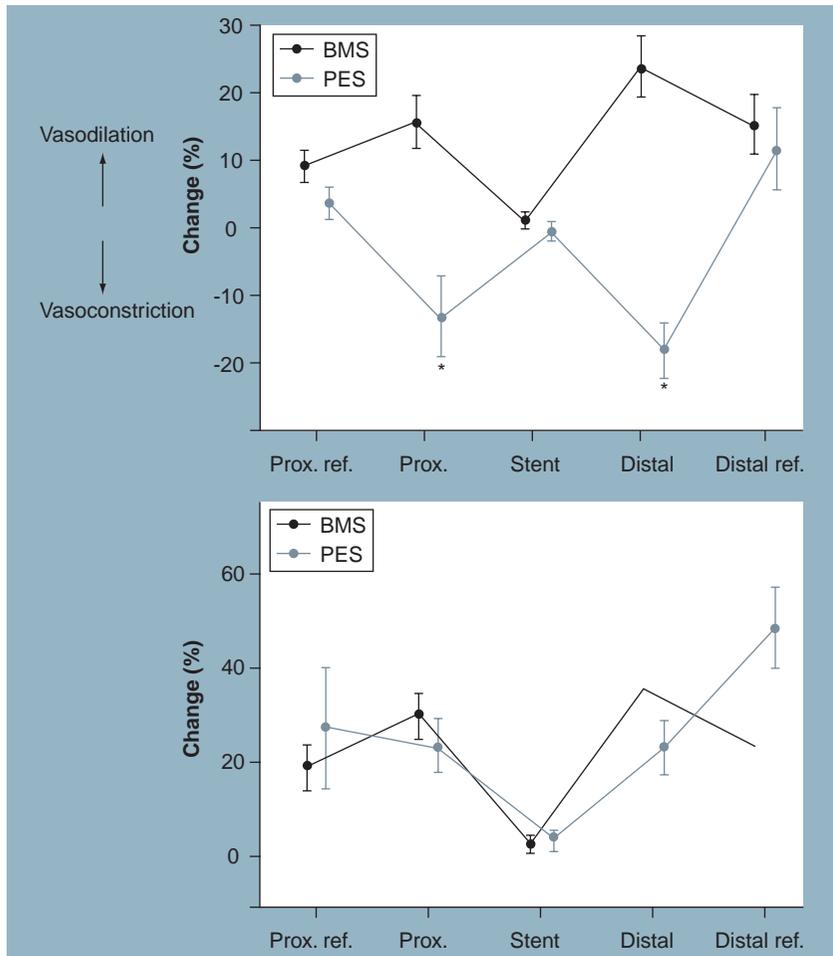
The zotarolimus-eluting stent (ZES; Endeavor™, Medtronic Vascular, Inc., CA, USA) has been reported to promote rapid and uniform healing of endothelium [40]. Recently, Kim *et al.* prospectively compared coronary endothelial function by measuring coronary vasoreactivity in response to titrated doses of intracoronary ACh and NTG in patients with ZES versus SES versus BMS implantation at 6-month follow-up [41]. A total of 55 patients who were diagnosed with stable angina and treated with a single stent for a *de novo* lesion of the left anterior descending artery were enrolled. Two segments in the study vessel were chosen for analysis, specifically, 5-mm proximal and distal to the site of stenting. Between groups, more intense vasoconstriction to incremental doses of ACh was observed in the DES groups at 6-month follow-up compared with BMS counterparts. Vasoconstriction to ACh was more prominent in the distal segments than the proximal

segments in both ZES and SES groups. On the other hand, endothelial function associated with the ZES was more preserved at 6-month follow-up compared with the SES (FIGURE 8). Endothelium-independent vasodilation to NTG did not differ significantly among study groups.

These observed differences between ZES versus SES in endothelial dysfunction could be due to several reasons. Obata *et al.* demonstrated that sirolimus released from SES reduced the level of VEGF in the coronary circulation 2 weeks after implantation [42]. An *in vitro* study by Jabs *et al.* reported that continuous sirolimus exposure causes impaired endothelium-dependent vascular relaxation by stimulation of mitochondrial ROS release [43]. Another mechanism might be earlier restoration of endothelial function after ZES implantation related to the more rapid elution of the active drug. Whereas in SES the loaded drug is released from the stent up to 60 days after stent



**Figure 6. Paclitaxel-eluting stent implantation is associated with exercise-induced vasoconstriction in the peri-stent region, suggesting endothelial dysfunction.** Original recording of the left circumflex artery at baseline (left), during exercise with 125 W (middle), and after 1.6 mg sublingual nitroglycerin (right). Right anterior oblique views are shown in the upper panels; the corresponding lateral views on the lower panels. The proximal and the distal segments adjacent to the two paclitaxel-eluting stents elicit severe vasoconstriction during exercise (arrows). After sublingual nitroglycerin, the proximal and distal segments dilate. Reproduced with permission from [9].



**Figure 7. Paclitaxel-eluting stent implantation is associated with exercise-induced vasoconstriction in the peri-stent region, suggesting endothelial dysfunction.** Percentage changes of cross-sectional lumen area in the control group (BMS) and paclitaxel group (PES), during exercise (upper panel) and after nitroglycerin application (lower panel). Mean values  $\pm$  standard error of mean are shown for the Prox. Ref.; the Prox.; the stent; the distal segment; and the Distal Ref. segment.  
 BMS: Biolimus A9-eluting stent; Distal Ref.: Distal reference; PES: Paclitaxel-eluting stent; Prox.: Proximal segment; Prox. Ref: Proximal reference segment.  
 Adapted with permission from [9].

implantation, ZES provides delivery of drug to the arterial wall only during the first 2 weeks of elution from the stent [44], and thus local toxicity is minimized. In addition, unique characteristics of the phosphorylcholine (PC) polymer could contribute to the differential effects of DES [41]. The PC polymer of ZES has, despite the permanent polymer, hydrophilic properties that are expected to generate less interfacial tension in the aqueous body environment and is thus more highly biocompatible compared with the hydrophobic polymer of SES, which has been shown to resist fibrinogen adsorption and cause less platelet and monocyte activation. Furthermore, the struts of ZES are thinner than those of the SES platform and previous reports demonstrated that reduced arterial injury and restenosis are associated with

thinner struts in humans [45]. The primary association has been the ability of endothelial cells to migrate across the thinner struts improving the rate of endothelialization *in vitro* [46]. In humans, ZES is associated with a greater amount of neointimal hyperplasia by IVUS at 8 months [47] and a homogeneous complete healing by OCT at 6 months compared with SES [48]. It can be speculated that, on the basis of OCT data and this study result, the more preserved endothelial function at 6 months in ZES could be associated with more complete endothelial coverage compared with SES. Whether this contributes to an improved safety profile in terms of late thrombosis is awaiting confirmation. Specific data regarding the direct relationship between endothelial function and healing over stent struts or clinical outcomes are lacking at this time. There is compelling clinical outcomes data that ZES carries an extremely low risk of late-ST [49].

Thus, despite the greater in-stent late loss, taken together, it is conceivable that a second-generation DES (ZES) could be more beneficial in specific clinical situations in which it is needed, for example, in patients who inevitably discontinue dual antiplatelet agents within 12 months as guidelines recommend due to scheduled surgery, or patients with questionable compliance for dual antiplatelet agents. However, these concepts will require further studies before implementation to daily clinical practice.

The Nobori stent™ (Terumo Corporation, Tokyo, Japan), is a different kind of second-generation DES that uses a bioresorbable polymer (polylactic acid) from which biolimus A9, an analog of sirolimus, is eluted. Biolimus A9 is a new sirolimus derivative that binds to cytosolic immunophilin FK binding protein 12. The formed complex binds to mTOR and inhibits growth-factor-driven cell proliferation. Its enhanced lipophilicity enables an increased uptake in local target tissues and reduced presence in areas surrounding the stented segment or even in the systemic circulation.

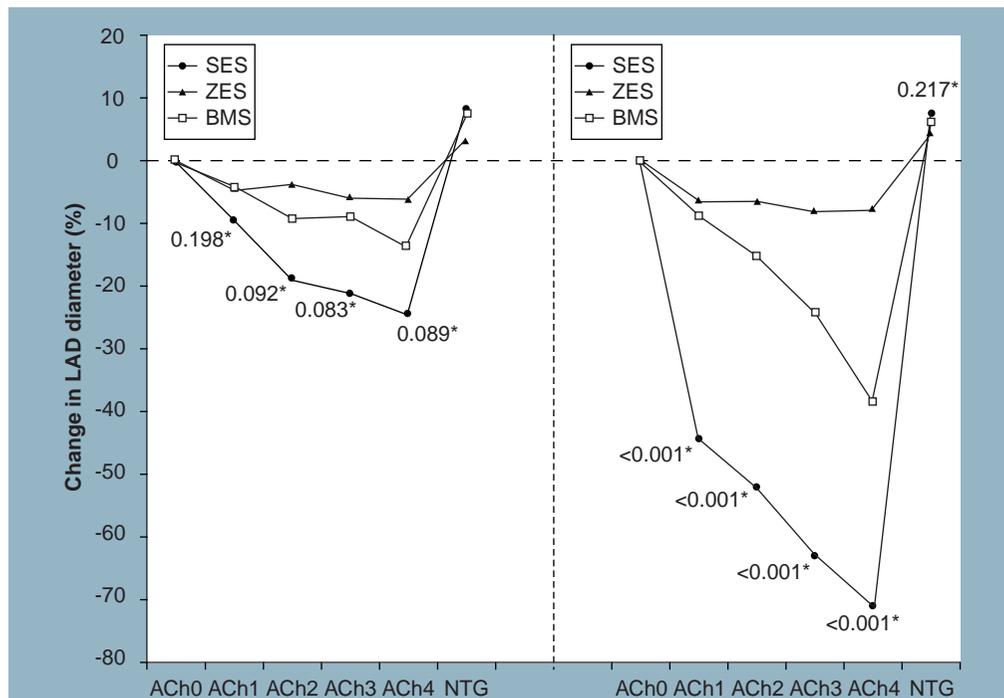
Hamilos *et al.* reported assessment of the endothelium-dependent as well as endothelium-independent coronary vasomotion 9 months after either biolimus A9-eluting stent (BES) or SES implantation, using right atrial pacing at increasing rate steps [50]. They found that unlike the case with SES, endothelium-dependent vasomotion at the adjacent stent segments appears to be well preserved after BES implantation (FIGURE 9). There can be several reasons why BES demonstrated better vasomotion response compared with SES. Biolimus A9 is more lipophilic than sirolimus,

and upon release would quickly bind to the target lipid-rich tissue. The drug is present only on the vessel side (abuminally), and as such enters into peripheral circulation in only minimal quantities [51]. This results in a more localized effect and less systemic drug exposure. Animal studies showed that after BES implantation, the tissue concentration of the drug in segments 5 mm proximal and distal to the stent edges is almost nonmeasurable [51] and stent endothelialization is more complete than with either SES or PES [52]. SES and BES have different drug-release kinetics; total drug content is released from SES within 60 days with more than 60% released shortly after stent implantation [51,52], versus a small initial burst and sustained simultaneous drug release and polymer degradation taking place over 6 months in BES [51], exposing the surrounding tissue at any given time to a lower amount of drug. Finally, the polymer used with BES is absorbed over a few months. Since durable polymers have been held responsible for some of the late adverse events related to SES, it is expected that the degradation of polymer will improve arterial healing and long-term safety of BES.

The fact that the majority of patients with BES had a vasodilatory response to pacing is a robust indication that functional endothelium regrowth is almost complete 9 months after implantation. The findings give promise that newer-generation DES with different drug and polymer platforms may be more respectful of the vessel healing while remaining at least as effective [53].

### Future perspective

Regardless of which mechanism(s) of the reduction of vascular function after DES deployment predominate, the following questions remain: what is the clinical relevance of these findings to late events and what can be done about it? In the literature, reduced endothelial function has been shown to correlate with increased atherogenesis, as well as cardiovascular risk [12]. Medical therapy including statins and ACE inhibitors have been demonstrated to improve endothelial dysfunction in patients with risk factors. Whether DES-induced endothelial dysfunction produces a similar risk is not known. However, in most long-term follow-up studies comparing the use of DES



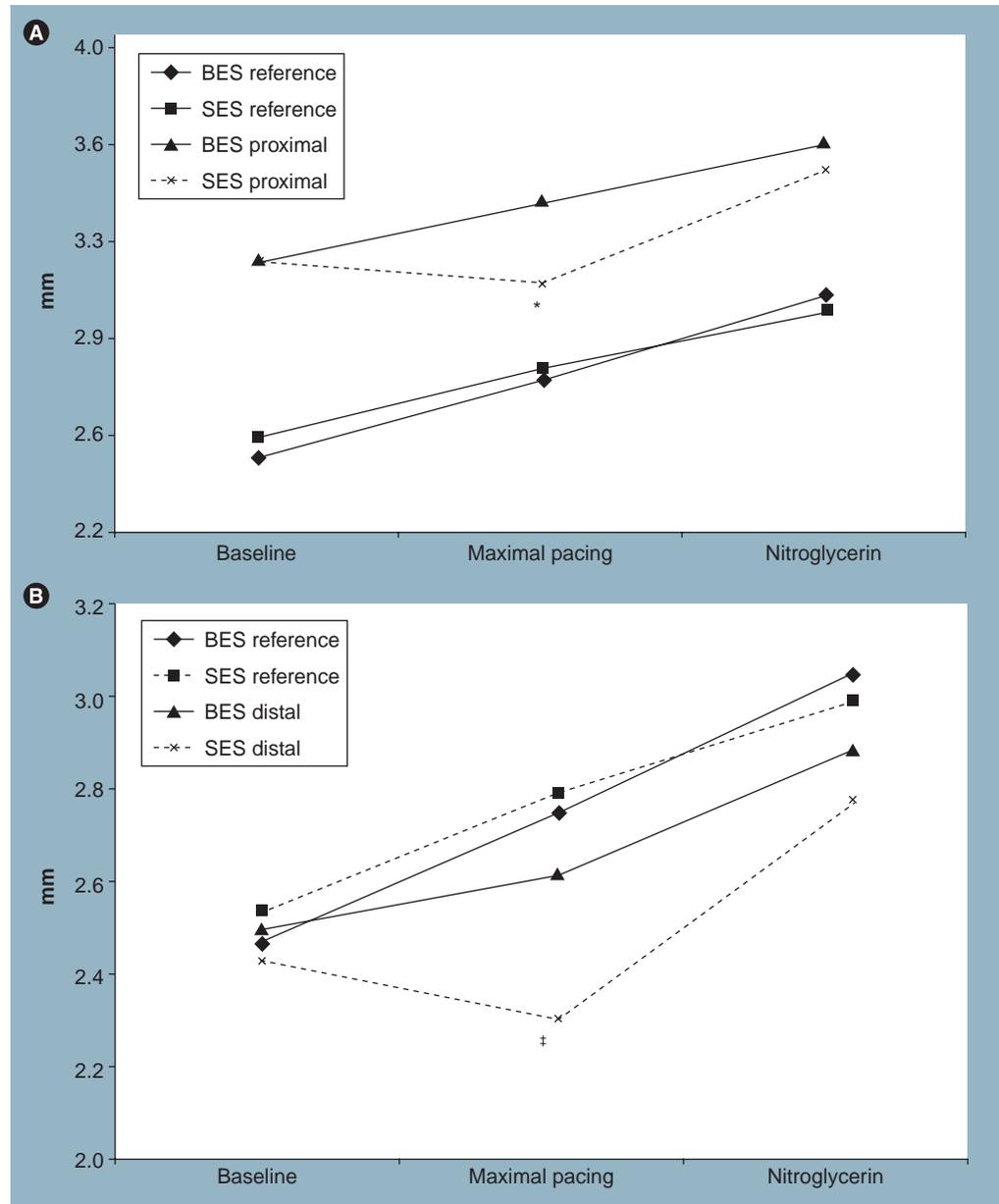
**Figure 8. Comparison of vasoreactivity between sirolimus-eluting stent, zotarolimus-eluting stent and bare-metal stent.**

\*p-value: SES versus ZES.

The diameter changes between the SES and the ZES had significant differences of vasoreactivity in response to the ACh1 to ACh4 doses in sites distal to the stents, but not the sites proximal to the stents. ACh1: 10 µg; ACh2: 20 µg; ACh3: 50 µg; ACh4: 100µg.

ACh: Acetylcholine; BMS: Biolimus A9-eluting stent; LAD: Left anterior descending coronary artery; NTG: Nitroglycerin; SES: Sirolimus-eluting stent; ZES: Zotarolimus-eluting stent.

Adapted with permission from [41].



**Figure 9. Mean diameter of the proximal, distal and reference segments in the biolimus A9-eluting stent and sirolimus-eluting stent groups.** Mean diameter values at baseline, maximal pacing rate, and after nitroglycerin administration in BES and SES groups, in proximal (A), distal (B) and reference (A & B) segments. The p-values for the comparison between BES and SES are indicated for both proximal and distal segments. BES: Biolimus A9-eluting stent; SES: Sirolimus-eluting stent. Adapted with permission from [50].

versus BMS, there is general lack of improvement in the hard outcomes of death and myocardial infarction [54]. Indeed, in several studies, the risk of death is increased with the use of DES over and above what might be expected from documented risk of ST [55]. It is possible that an increased cardiovascular risk from DES-induced endothelial dysfunction may help to explain these findings. In addition, it is possible that DES-associated endothelial dysfunction may be generalized to other coronary arteries aside from the specific

one where the DES was placed. Finally, if DES-associated endothelial dysfunction is a real and harmful phenomenon, what can we do about it?

### Conclusion

In summary, DES-associated endothelial dysfunction is reproducible and persists for an extended period of time after the stent implantation. Further studies are indicated to clarify its clinical significance and potential correlation to late events.

**Executive summary**

- First- and second-generation drug-eluting stents (DES) are widely used in interventional cardiology and they significantly reduce the rate of target vessel revascularization.
- Despite their widespread use, there is limited information regarding the long-term effects of DES on endothelial function and vascular healing.
- Here we review several studies in humans in the cardiac catheterization laboratory following DES implantation that show impaired endothelial functioning in both the proximal as well as distal stent segments.
- Although long-term effects of DES-induced endothelial dysfunction are unknown, they require further investigation for their potentially adverse vascular and possible clinical events.

**Financial & competing interests disclosure**

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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