Summary and conclusions
SUMMARY AND GENERAL DISCUSSION

Constant vasodilatation, inhibition of platelet and monocyte adhesion, and local thrombolysis are the mechanisms through which an intact endothelial layer protects coronary vessels from injuries and maintains an adequate myocardial perfusion. Subsequently a loss in these features is considered to be the first step in the development of atherosclerosis, and a potent trigger for complications after revascularisation procedures. Chapter 1 deals with the state-of-the art about endothelial and platelet pathophysiology in the course of coronary atherosclerosis, with a particular regard for the alterations induced by percutaneous and surgical revascularisation on endothelial function and on the process of endothelium-platelet interaction. This comprehensive review also addresses behavioural and pharmacological interventions able to revert early endothelial dysfunction, as well as the wide spectrum of agents for preventing platelet aggregation and endothelium-platelet interaction in the course of percutaneous coronary interventions.

Taking as a starting point the recent results of the AVERT trial, which demonstrated a superiority of Atorvastatin vs. percutaneous interventions in preventing cardiac events in a population with mild coronary artery disease, chapter 2 is entirely dedicated to debate pros and cons of medical therapy vs. revascularisation in the treatment of coronary artery disease. Indeed, statin therapy appears particularly effective not only in reversing plaque formation and in promoting plaque stabilisation, but also in improving endothelial function, so that it can be offered either as an effective alternative to revascularisation in the treatment of early coronary atherosclerosis or as a complementary treatment for late coronary atherosclerosis.

If the presence of coronary atherosclerosis is suspected, and angiography has ruled out the occurrence of flow-limiting stenosis, premature endothelial dysfunction can still be discovered. An impairment in endothelium-dependent vasomotility can be easily assessed by selective coronary infusions of acetylcholine, which, when not counteracted by the endothelial release of nitric oxide, induces a net vasoconstriction. As demonstrated in chapter 3, coronary angiography itself can be considered as a provocative test for endothelium-dependent vasomotility. In our study group of patients with suspected coronary atherosclerosis, non-ionic contrast media (iodixanol and iopromide) induced a vasodilator response in normal coronary vessels, not by a mechanism involving increased flow or endothelial nitric oxide synthesis, but rather by depending on preserved vascular cyclooxygenase activity. Instead, a ionic agent (ioxaglate) had no significant effect on coronary vasomotility. In the presence of coronary artery disease normal epicardial vasodilator response is changed into vasoconstriction. Also in non-stenotic segments of diseased vessels vasoconstriction can be found, thus confirming that endothelial dysfunction is a widespread phenomenon, which can be present before and eventually contribute to the development of an angiographically-evident stenosis. Our results can also have important relapses on the setting of percutaneous coronary interventions. First of all, given the lower activity of ionic than non-ionic agents in stimulating vasoconstrictive responses, the former may reduce vasospastic-related ischemic events during coronary interventions. The use of inert contrast agents should also be appointed to improve the accuracy of on-line quantitative measurements.
(reference diameters, stenosis percentages) before intervention and the reliability of angiographic assessment in course of provocative tests for coronary vasomotion. Any externally-induced interference is supposed to result in unpredictable effects on the coronary homeostasis. More than coronary angiography, revascularisation procedures can provoke a rugged worsening of a pre-existing impairment in endothelium-dependent vasomotility. Chapter 4 describes that, either through a mechanical stimulation of the surrounding vessel wall or due to direct plaque disruption, percutaneous coronary interventions induce an acute and pronounced local release of endothelin-1. The greater release of this molecule after stent deployment is suggestive for a more severe acute vascular injury than after balloon angioplasty. Endothelin-1, a potent endothelium-derived vasoconstrictor and growth mediator, can induce unwanted vasoconstriction, leading to epicardial vasospasms or microvascular no-reflow phenomenon immediately after intervention.

Coronary stents are also entitled to prime chronic endothelial dysfunction throughout a process of inflammation at the site of deployment, which extends over time after intervention. In a population of patients successfully submitted to coronary stenting (Chapter 5), we demonstrated that vasoconstriction to acetylcholine was present at six months after intervention, both in the distal segments of the treated arteries and of the controlateral non-stenotic vessels. This finding confirms that dysfunctional endothelium and flow-limiting stenosis are two independent events in the course of atherosclerosis. However, and more clinically relevant, only the occurrence of impaired endothelium-dependent vasomotility in the stented, but not in the controlateral vessel, was correlated with subclinical ischemia at follow-up. Impaired endothelial vasomotility following stenting may involve epicardial, as well as microvascular vessels. By a sustained release of vasoactive substances and inflammatory mediators stents can influence the distal vessel bed. Therefore, they appear at best ineffective in improving, if not responsible of worsening, a pre-existing impaired endothelium-dependent vasomotility. The subclinical ischemia could be driven by a reduction in epicardial or microvascular vasodilative reserve or by vasospasms. Yet, it seems of crucial importance to minimise both the acute mechanical trauma and the chronic vessel inflammatory responses following coronary stenting.

Since the moment of their first utilisation, new models of coronary stents have been continuously developed, in order to improve immediate and long-term outcomes of percutaneous revascularisation. Biotechnical researchers have recently acknowledged the importance of hemocompatibility in predicting stent success after coronary implantation. By means of an original experimental model for blood circulation (Chapter 6), we proved how a stent of new-generation (MultiLink), when compared to traditional stainless steel models, triggers almost no activation of the coagulation system, and a significantly lower platelet and leukocyte deposition. In fact, geometry, design and shape can dictate different hemocompatibility, even when devices are made from the same material. In the clinical setting, this reduced interaction of the stent surface with platelets, leukocytes and coagulation cascade is expected to translate into a reduced occurrence of acute thrombosis and late restenosis. However, as coronary stents represent metal foreign bodies, directly able to promote platelet activation and thrombosis, the contemporary use of antiplatelet agents is still recommended when performing coronary stenting.
Nevertheless, our study suggests that, when satisfactory hemocompatibility is provided, any aspirin-like drug may be sufficient in the course of routine procedures. A broader range of interventional settings, during which platelets have to be aggressively tackled, is lately emerging (primary stenting for acute myocardial infarction, irradiation therapy for in-stent restenosis). In these cases a more effective antiplatelet regimen than conventional antiaggregant therapy is probably required. In a second study with the same experimental model, an in-vitro comparison of different antiplatelet agents for the prevention of stent thrombosis was performed (chapter 7). The association of an aspirin-like treatment with a front-load dose of Clopidogrel, an ADP-receptor antagonist, which is the conventionally-accepted antiplatelet regimen, provided less satisfactory results than platelet GpIIbIIIa receptor inhibitors, Abciximab and Eptifibatide, which, on the other hand, proved an equal efficacy in preventing platelet aggregation and deposition on stainless steel surfaces. From our study also emerged that circulating platelets from patients affected by coronary atherosclerosis can exhibit an overexpression of GpIIbIIIa receptors, responsible for a manifested interindividual variability to antiplatelet treatment, that can be counteracted only by an aimed platelet receptor blockade. Besides, the GpIIbIIIa inhibitor Abciximab can also prevent the adhesion of circulating elements (platelets, leukocytes) onto endothelial cells, so it can reduce the development of the chronic inflammatory process, and may have long-term beneficial relapses, after percutaneous coronary interventions.

In our opinion, not only interventional cardiologists, but also cardiac surgeons, should take an interest in the study of vascular endothelial function. In fact, according to and extending previous findings of other authors, we confirmed that the maintenance of a preserved endothelial function after surgery is the best feature of arterial conduits, specifically of internal thoracic arteries, when used as in-situ coronary grafts. In chapter 8 we demonstrated that the physiological vasodilative response to acetylcholine of the left internal thoracic artery, long term after being grafted, is fully preserved. Though both endothelium-dependent and independent vasodilative pathways of the graft do not deteriorate over time after surgery, nevertheless they depend on the age of the patient, probably for a process of ageing involving the media layer.

The anatomical and functional characteristics of the internal thoracic artery late after surgery strongly support the continued use of arterial grafts regardless of the presence of multiple risk factors and severe atherosclerosis. Nevertheless, giving confirmation for the detrimental effects of smoking on endothelial function, our results presented in chapter 9 demonstrated that, among all the coronary risk factors, cigarette abuse continued after, but not preceding surgery, appeared the only predictive factor for a reduction in endothelium-dependent vasodilatation in internal thoracic artery grafts. Whether this finding might negatively influence the long-term outcome has to be investigated, but an effort should be made in convincing patients who undergo surgical revascularisation of the beneficial effect of quitting smoking with the aim of preserving the good functionality of the implanted grafts.
CONCLUSIONS AND CLINICAL IMPLICATIONS

From a comprehensive overview of our results clearly emerges that, in patients suffering from coronary artery disease, an altered endothelium-dependent vasomotility and an activation of circulating platelets are already present before attempting coronary revascularisation, due to the evolving atherosclerotic process and the influence of risk factors. While percutaneous interventions can induce further endothelial dysfunction throughout acute mechanical and chronic inflammatory triggers, on the other hand the solely use of *in-situ* arteries during bypass surgery seems not to alter the endothelial functional integrity of grafted conduits.

As revascularisation procedures, especially coronary stenting, stimulate circulating platelets to aggregate, the combination of the two pathological phenomena, endothelial dysfunction and platelet activation, and the subsequent dysregulation of endothelium-platelet interaction, if not effectively counteracted, can negatively and markedly influence the outcome of revascularised patients.

Assuming that the continuous increase of revascularisation procedures, that we are facing now-a-days in Western Europe, is functional for an effective treatment and care of ischemic patients, nevertheless we are convinced that, based on the mounting pathophysiological knowledge, also supported by our present findings, a sole mechanical approach to coronary stenosis is limiting. We argue that it should be integrated in a more complete treatment, that is also aimed at the metabolic and biocellular processes, of the coronary atherosclerotic disease *in toto*. In our opinion, some recommendations are mandatory in order to optimise the immediate and long-term outcomes of revascularisation procedures and to control, when not to defeat, coronary atherosclerosis:

1. To prevent the harmful impact of coronary risk factors. Health-care policies such as low-fat diet, active lifestyle, but particularly a firm campaign against cigarette abuse, should be strongly pursued independently from revascularisation, as their beneficial impact on endothelial and platelet function will reverberate on quality of life and long-term prognosis.

2. To minimise the impact of revascularisation procedures on vascular physiology. Interventional cardiologists should guard against an indiscriminate use of coronary stents, and, when necessary, prefer devices with features of enhanced hemocompatibility. The utilisation of vasomotor-inert contrast media during cardiac catheterisation should also be encouraged. Cardiac surgeons should invest in strategies aimed at preserving the grafted conduits in their physiological status: minimally invasive surgery, exclusive *in-situ* arterial grafts, prevention of technique-related vasoconstriction during graft preparation.

3. To supply specific and adequate pharmacological support before, during and after intervention. Especially statins, but also ACE-inhibitors, proved to be helpful in restoring physiological endothelial conditions, in the systemic, epicardial, and microvascular territories, and put themselves forward as a necessary complement for an effective myocardial revascularisation. GpIIbIIIa inhibitors appear fundamental in course of percutaneous coronary interventions, particularly after stenting, for inhibiting both platelet activation and endothelium-platelet interaction, and interrupting the course of acute and chronic vascular responses.