Endothelial Therapy of Atherosclerosis and its Risk Factors

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Abstract: Atherosclerosis is a chronic systemic disease of the vasculature with an inflammatory component. It accounts for the majority of cardiovascular morbidity and mortality in industrialized countries and its incidence is increasing in developing countries. The impairment of vascular endothelial cell function in atherosclerosis and in conditions associated with increased cardiovascular risk is an important determinant of disease progression. The reduction of endothelium-dependent relaxation in the coronary and systemic circulation in atherosclerosis is in part due to decreased bioavailability of nitric oxide and increased release of oxygen-derived free radicals. Atherosclerosis also increases the formation of vasoconstrictors and growth factors, adhesion of leukocytes, thrombosis, inflammation, cell proliferation, as well as increases in vascular tone. Here we review mechanisms and therapeutic approaches to improve endothelial pathways in atherosclerosis. Restoration of NO bioactivity through pharmacological inhibition of the renin-angiotensin system, statin therapy, or endothelin receptor blockade, ameliorates vascular function in experimental hypercholesterolemia, hypertension and heart failure. These treatments also have therapeutic benefit for patients at risk or with overt atherosclerosis, to reduce vascular and myocardial complications of this disease.

Keywords: ACE inhibitors – atherosclerosis – endothelium – endothelin – nitric oxide – risk factors – statins – vascular

ATHEROSCLEROSIS – A CHRONIC INFLAMMATORY VASCULAR DISEASE

Atherosclerosis is a chronic inflammatory systemic disease of the vasculature which forms the basis for most cardiovascular and renal diseases such as coronary artery disease, congestive heart failure, peripheral artery disease, stroke, ischemic bowel disease [1-4], and diabetic nephropathy [5]. Diseases caused by atherosclerosis still account for the majority of morbidity and mortality in industrialized countries [6] and the incidence of atherosclerosis is rapidly rising in developing countries [7] [8].

Early atheromatous vascular changes such as the "fatty streak" are the beginning of a chronic process which is associated with activation of inflammatory pathways within the vessel wall. The fact that "fatty streaks" are already detectable in the fetal aorta and aggravated by maternal hypercholesterolemia [9] is reason enough to consider medical treatment of risk factors at a much earlier timepoint than we currently do. In fact, a recent study suggests that even the treatment of common atherosclerotic risk factors is often underestimated by physicians [10].

The number of risk factors implicated in the atherogenic process suggest that a multitude of mechanisms and factors is involved in the development of atherosclerosis [2]. According to Russell Ross, a "response to injury" of the endothelium promotes disease progression [11,12], which can be viewed as a pathophysiological reaction to different risk factors sharing "proinflammatory" stimulation of cells in the vessel wall. Risk factors are aging, estrogen deficiency, elevated LDL cholesterol, low HDL cholesterol levels, hypertension, hyperglycemia and diabetes, obesity, smoking, physical inactivity, genetic variability, and chronic inflammatory processes (reviewed in [2,3]). A role for infection due to influenza [13,14], herpesviruses, chlamydia pneumoniae [15,16], and nanobacteria [17] has been postulated, however, the evidence for a causal role of these agents is still lacking [16]. The vascular injury process, which is associated with an increased endothelial permeability for lipoproteins, enhances the local release of vasoactive factors such as nitric oxide (NO), prostacyclin, angiotensin II [18], endothelin, platelet derived growth factor, tumor necrosis factor-α, and interleukin-1β (reviewed in [1-3]). Furthermore, upregulation of cell adhesion molecules including PECAM-1, VCAM-1, ICAM-1, selectins, and integrins occurs as well as migration of leucocytes into the vessel wall. These changes are further enhanced by oxidized-LDL, MCP-1 [19], MCSF, IL-8 [19], PDGF, and osteopontin [20-22] (all reviewed in [2]).

If the inflammatory response is maintained by continued stimulation, migration and proliferation of VSMCs results and VSMCs will finally change their phenotype. These VSMCs cover accumulated lipids ("lipid core") as well as inflammatory cells, such as macrophages and lymphocytes, forming the so-called "fibrous cap" [23]. For the disease process thereafter, it becomes crucial whether the cap of the plaque remains covered by VSMCs ("stable plaque") or thinning of the fibrous cap occurs. Rupture of a "vulnerable plaque" leads to local activation of clotting factors,

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thrombosis, and infarction downstream of the rupture site [24,25].

THE PROATHEROGENIC AND ANTIATHEROGENIC POTENTIAL OF ENDOTHELIAL CELLS

According to current concepts, functional alterations of the endothelium play a key role for the development of atherosclerosis. These alterations are usually referred to endothelial "dysfunction", a term which cannot be clearly defined. This term has been used by investigators to describe impaired vasodilation to acetylcholine or a decreased bioavailability to NO in experimental models and also in humans [26,27]. With increasing knowledge in the area of vascular biology, it became clear that many factors are involved in the physiological functions of the endothelium, and thus contribute to pathological alterations resulting in endothelial "dysfunction". In healthy blood vessels, the production and bioactivity of endothelial factors is balanced, whereas disturbances of this balance, triggered by the aforementioned cardiovascular risk factors, increase the release of growth-promoting and vasoconstricting mediators such as angiotensin II and endothelin-1 (reviewed in [28,29]), whereas the bioavailability of vasodilating substances (e.g. NO) decreases [30] (Fig. **1**). Therefore, endothelial cells may play both a pro- and antiatherogenic role in this process. Over the past two decades, researchers have investigated whether and by what mechanisms endothelial factors contribute to atherogenesis. A number of studies have reported increased decomposition of NO by superoxide anion [31] due to increased oxidative stress in conditions associated with cardiovascular risk [32,33], and similar observations have been made in animals and patients with overt atherosclerosis [34-33]. Moreover it has become obvious, that angiotensin II is a potent pro-inflammatory substance [35,36] which generates reactive oxygen species (ROS) by activating NADPH oxidase [32,33] and

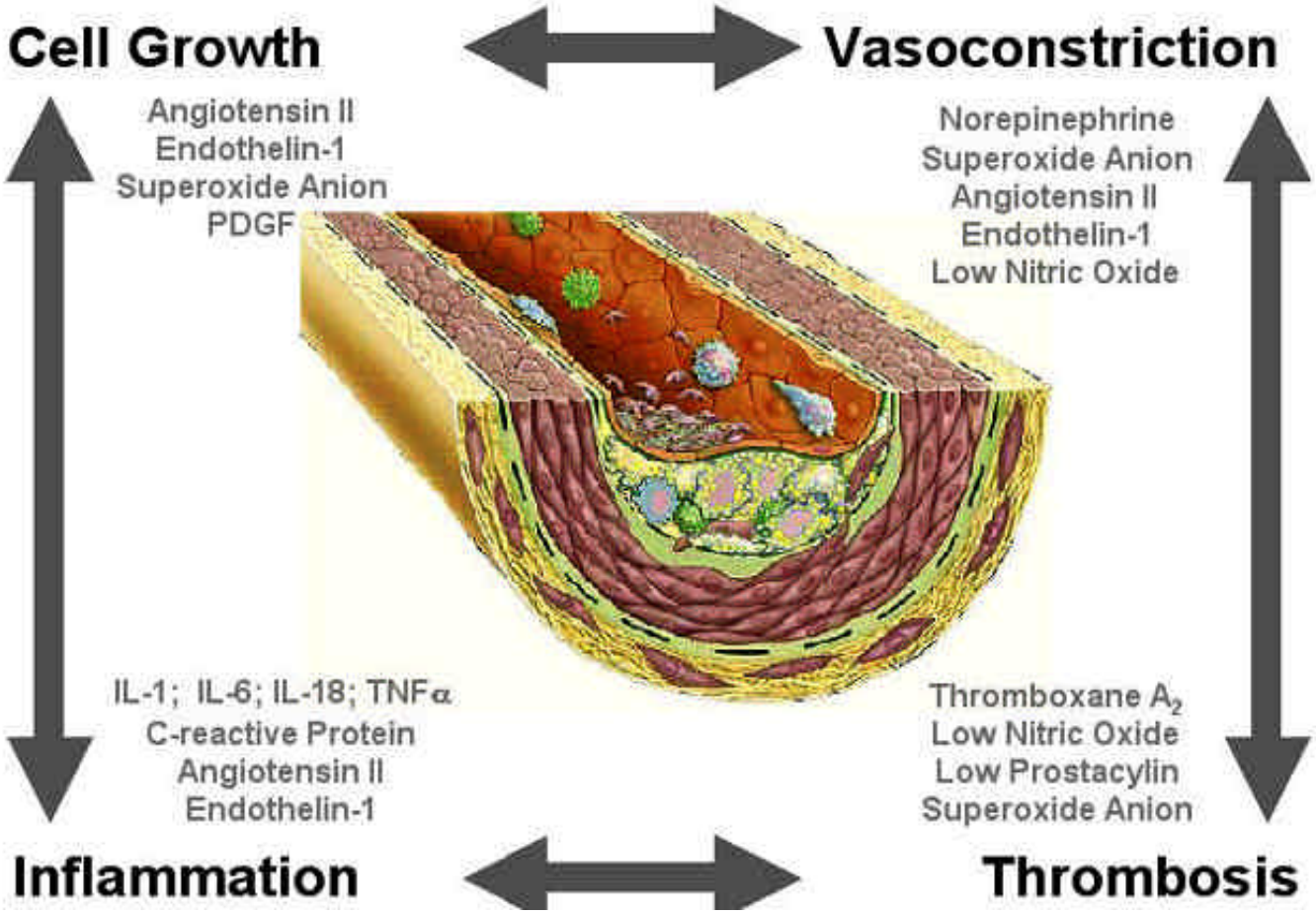

Fig. (1). This figure depicts the interactions of four major cardiovascular processes, namely inflammation, cell growth, vasoconstriction, and thrombosis. Inflammation appears to be one of the key events in atherogenesis, unifying the three other processes, since in the early stages of the disease inflammatory activation of monocytes occurs, which then become resident macrophages. Subsequently, macrophages release cytokines and other signalling molecules such as superoxide anion which then trigger a number of mechanisms leading to proliferation of VSMC. A number of these factors with mitogenic properties also act on vasomotion, increasing vascular tone. Vasoconstriction further aggravates thrombosis, which is the cause of myocardial infarction following plaque rupture (partly adapted from [2]). Abbreviations: IL-1, interleukin-1; IL-6, interleukin-6; IL-18, interleukin-18; PDGF, platelet-derived growth factor; TNF-α, tumor necrosis factor-α.

vasocontrictor prostanoids [37]. Several studies have shown that angiotensin II directly promotes atherosclerosis in this fashion (reviewed in [18]), and that it also contributes to the formation of atherosclerotic aneurysmens [38-41].

CARDIOVASCULAR RISK FACTORS ALTER ENDOTHELIAL CELL FUNCTION

Furchgott and Zawadzki initially described that the vascular endothelium regulates vascular smooth muscle cell tone by demonstrating that in the isolated rabbit aorta removal of endothelial cells abolishes the relaxation in response to pharmacological stimulation with acetylcholine [42]. These experiments suggested the existence of an endothelium-derived relaxing factor (EDRF) [42], acting through cGMP [43], which was subsequently identified as nitric oxide (NO) [44]. Since then, other actions of NO such as control of cell growth, blood cell-endothelial cell interactions, immunomodulation, and modulation of the clotting system have been described (reviewed in [34]).

The reduction of NO bioactivity is mainly due to

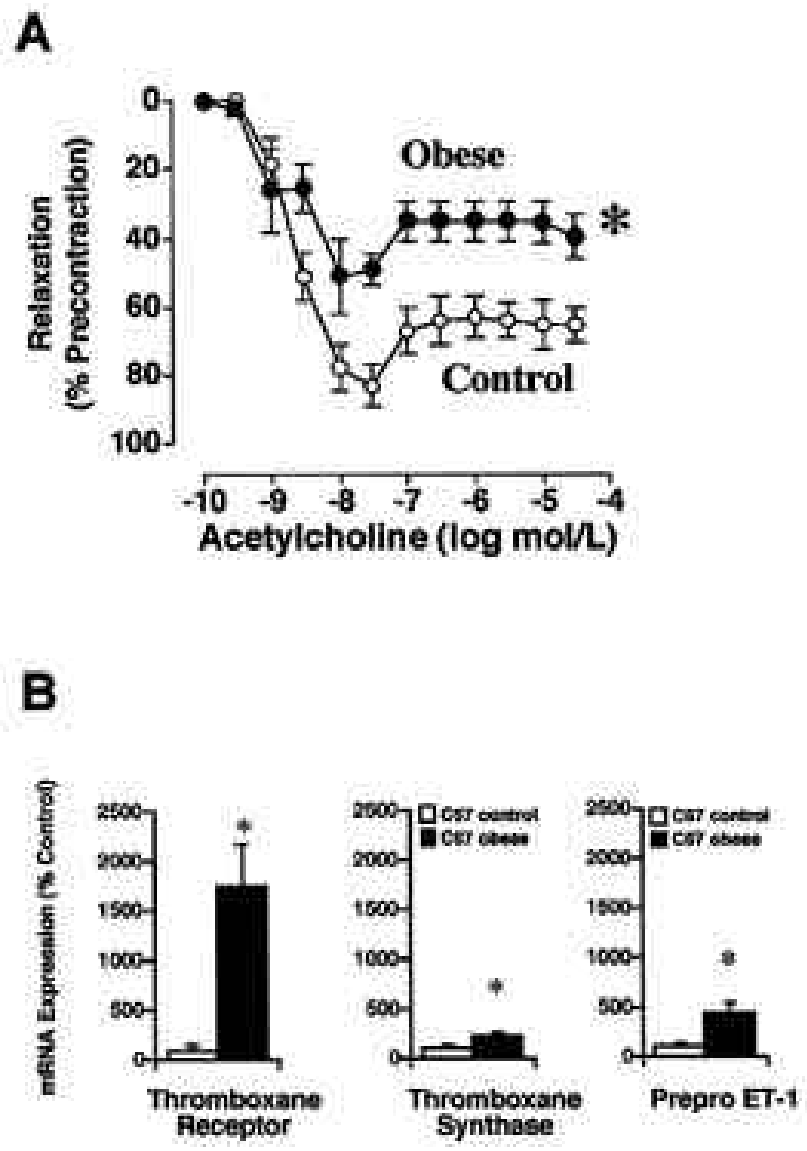

Fig. (2). Effects of obesity on endothelium-dependent vascular function and gene expression in mouse carotid artery. (A) Dose response curves to acetylcholine in isolated mouse carotid artery rings precontracted with norepinephrine. In control mice, relaxation to acetylcholine was antagonized by a contractile response at high concentrations. In animals that were treated with a high-fat, Western-type diet (42% from fat, 0.15% cholesterol), the relaxant response was significantly attenuated and the contractile portion of response was enhanced ($p<0.05$). (B) Gene expression as determined by real-time quantitative PCR in mouse carotid arteries. Obesity was associated with a greater than 18-fold increase of thromboxane receptor expression, whereas the increase in thromboxane synthase and prepro ET-1 mRNA expression was less pronounced. Of note, animals were normotensive and did not show macroscopic atherosclerotic disease ($p<0.05$, reproduced from [59]).

mediators such as angiotensin converting enzyme or endothelin [80,81,97]. It is, therefore, not surprising that drugs initially designed to correct hyperlipidemia such as statins have also been shown to reduce blood pressure in animals and humans [99,100]. Indeed, a therapeutic benefit with these lipid-lowering compounds is observed also in patients with so-called "normal" cholesterol levels, as shown in the Air Force/Texas Coronary Atherosclerosis Prevention Study [101,102]. Similarly, many drugs which were initially designed as antihypertensives such as ACE-inhibitors, AT_1-antagonists, calcium channel blockers, and ET antagonists have been shown to have profound effects on vascular structure [80] that cannot be explained by the drugs' antihypertensive effects alone. Therefore, the powerful "class-independent" actions on vascular cell function and structure of many of today's cardiovascular drugs should be considered when treating patients with or at risk for atherosclerosis. Also, one should remember that inhibitors of the renin-angiotensin system, in addition to their antihypertensive effects, have a number of pressure-independent effects on renal [103-105] and myocardial structure [106]. Moreover, there may be additive effects of well established cardiovascular drugs, as some recently published studies suggest [107-109].

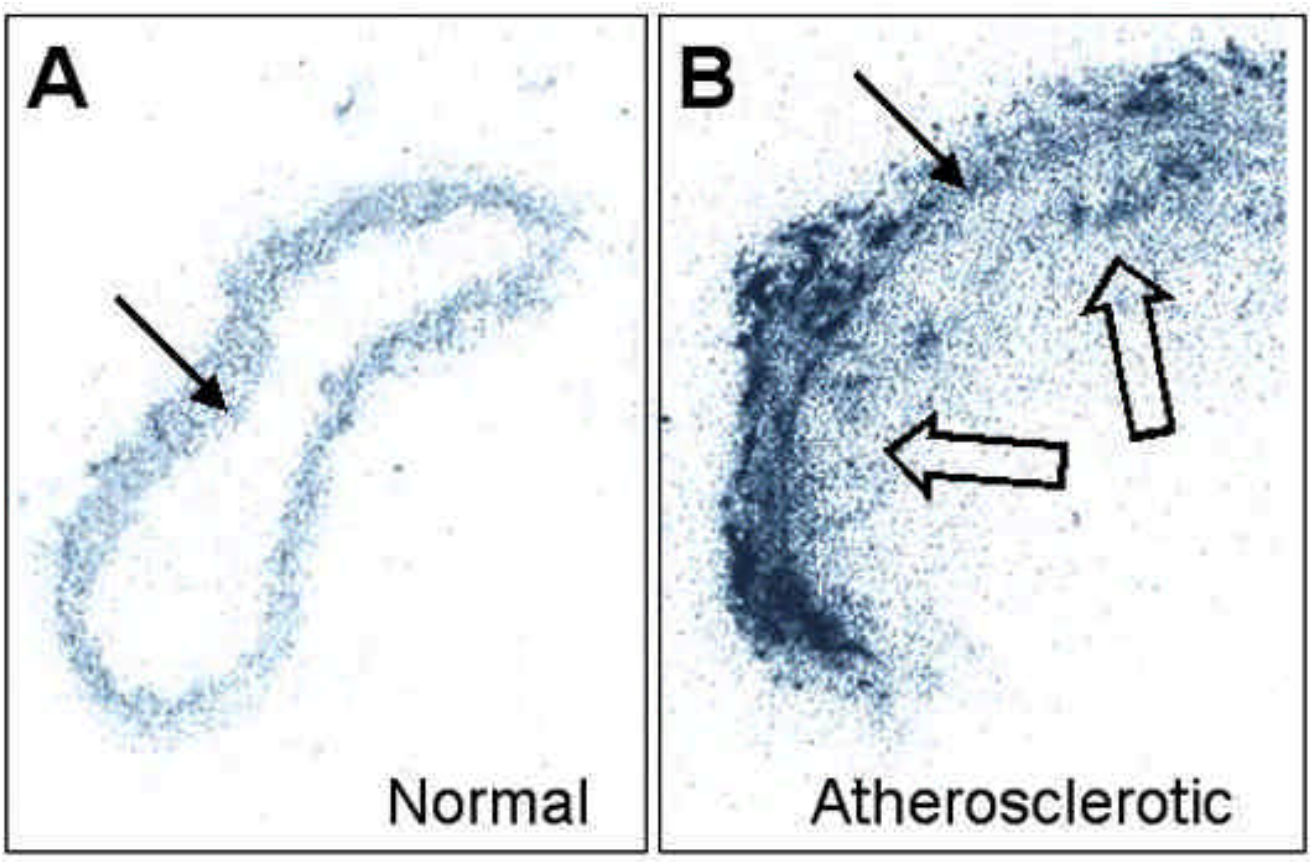

Fig. (3). Autoradiographs of radioactively labelled ET-1 in the mouse vasculature. (A) Shown here is a cross-section from a thoracics aorta obtained from a healthy C57BL/6J mouse which has been labelled with ^{125}ET-1. Binding of ET-1 is present in the entire vessel wall and shows a homogeneous distribution. (B) Section of a thoracic aorta of an 8-month old apoE-/- mouse treated with western-type diet showing an advanced atherosclerotic lesion. Compared to the C57BL/6J mouse, binding of radiolabelled ET-1 is markedly increased and not only confined to the media (solid arrows), but can also be detected in the area of the atherosclerotic lesion (open arrows) (modified from [76]).

ATHEROSCLEROSIS AND INFECTION: CAUSE OR CONSEQUENCE?

Clinical and experimental studies in recent years have also added new cardiovascular risk factors, which may be new targets to therapeutic intervention, such as chronic inflammation, possibly infectious microorganisms [15], or even elevated CRP serum levels [110,111]. This may be even more important since elevated CRP levels are a sensitive predictor of cardiovascular events [110,111]. Several studies have investigated treatment of an "infectious burden" in patients with cardiovascular diseases [112-115], but a convincing therapeutic strategy as well as a mechanistic link between one single infectious agent and the development of atherosclerosis still remains to be demonstrated [16]. However, the relationship between elevated CRP serum levels and cardiovascular risk is undisputable and seems to be a stronger long term-marker for cardiovascular mortality than LDL plasma levels [111] and possibly a novel therapeutic target [116,111]. Indeed, CRP not only serves as an inflammatory marker, but has distinct effects on endothelial cells as well as on white blood cells [117]. C-reactive protein reduces the expression of eNOS and NO bioactivity in cultured endothelial cells [118]. Furthermore, increased CRP inhibits angiogenesis, possibly through a reduction NO production [119]. In this context however, it should be noted that angiogenesis enhances atherosclerosis, at least in certain experimental models [120]. Therefore, it is currently impossible to determine whether angiogenesis is beneficial or detrimental to atherogenesis in humans. However, CRP has been shown to increase monocyte adhesion to human endothelial cells, suggesting that increased levels of CRP in patients with atherosclerosis could contribute to foam cell formation [117]. Interestingly, CRP also could have anti-atherosclerotic properties as it is a vasodilator in human arteries [121].

PRACTICAL ASPECTS OF ENDOTHELIAL THERAPY

In addition to pharmacological approaches and perhaps most importantly, we need to increase the awareness for the factors triggering and aggravating the development of atherosclerosis not only in the general population, but also among physicians [10]. This can be best achieved by an information policy provided by specialized physicians that allows the individual to recognize the importance of risk factors, their early impact on vascular alterations and diseases

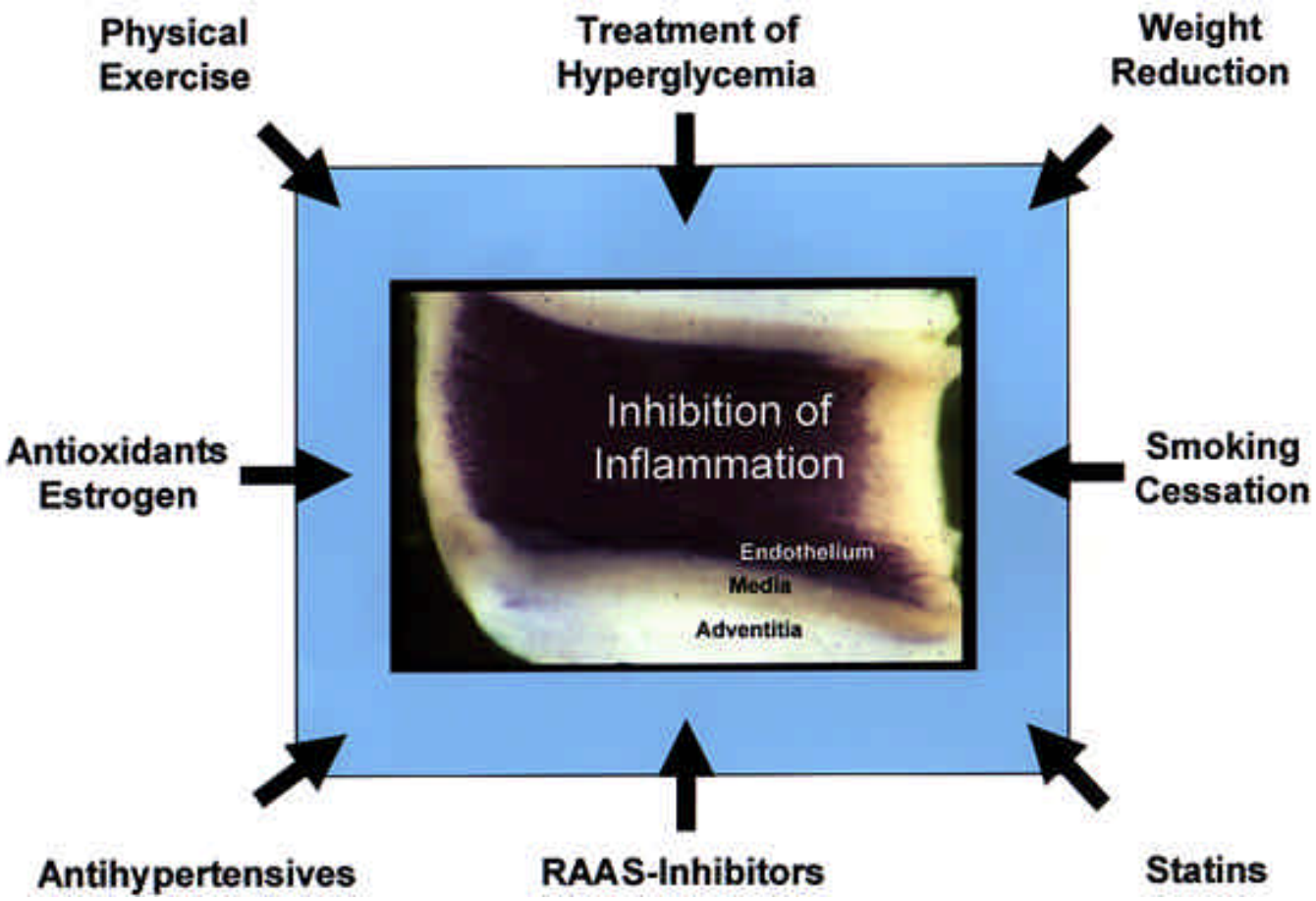

Fig. (4). Modalities to interfere with vascular inflammation to improve endothelium-dependent vascular function. Shown here is a macroscopic photograph of a healthy porcine coronary artery in which the endothelial cells were visualized by histochemical methods. The vessel has been opened longitudinally and cut in half. Endothelial cells are aligned in the direction of blood flow. The three different vascular layers endothelium, media, and adventitia can be easily recognized. As shown here, a number of non-medical and medical interventions can interfere with vascular inflammation, thereby reducing "dysfunction" of the endothelial cell and inflammatory responses. These interventions include physical exercise, cessation of smoking, and weight reduction as important lifestyle changes; furthermore certain antioxidants, estrogen, antihypertensives, inhibitors of the renin-angiotensin system, statins, and treatment of hyperglycemia with diet or using antidiabetic drugs ameliorate impairment of endothelial cell function. These measures, which have been shown to inhibit atherosclerosis in experimental models, are likely to provide therapeutic benefit in patients with the atherosclerosis and to slow down progression of this multifactorial disease.

which will develop. Even more important, we need to convince our patients about the possibility to interfere with and to slow down many of the pathological consequences if preventive measures are met early (Fig. **4**). A single and very powerful way of prevention is lifestyle modification, including cessation of smoking, correcting eating habits, regular physical exercise [122-124] and maintaining normal body weight and/or reducing obesity [125,126] since all of these lifestyle changes improve endothelium-dependent vasomotion. These possibilities as well as continued awareness of the patient`s blood pressure levels and plasma cholesterol values by the patient himself need to be further incorporated into our societies. Because of the option of reducing endothelial cell-related injury (Fig. **4**), these non-medical approaches may actually provide greater benefit for the prevention and treatment of atherosclerosis than we can currently appreciate. In view of the increased incidence of childhood diseases, including obesity [127-129], this awareness should particularly be communicated to parents and teachers to avoid disease in adulthood.

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ABBREVIATIONS

ACE = Angiotensin converting enzyme

EDRF = Endothelium-derived relaxing factor

ET	=	Endothelin
ICAM-1	=	Intracellular adhesion molecule-1
IL-1/-8	=	Interleukin-1/-8
LDL	=	Low density lipoprotein
MCP-1	=	Monocyte chemoattractant protein-1
MCSF	=	Macrophage colony stimulating factor
PDGF	=	Platelet-derived growth factor
PECAM-1	=	Platelet-endothelial cell adhesion molecule-1 (CD31)
TNF-α	=	Tumor necrosis factor-α
VCAM-1	=	Vascular cell adhesion molecule-1
VSMC	=	Vascular smooth muscle cell

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