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MINIREVIEW

Cholesterol Lowering and the Vessel Wall: New Insights and Future Perspectives

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Summary

The basis for most acute coronary events is either rupture or fissuring of unstable atherosclerotic plaques with subsequent thrombosis leading to coronary artery occlusion. The development of atherosclerotic plaques takes several decades, but the mechanical features determining its stability and the risk of rupture can change very rapidly depending on a number of internal factors. Unstable plaques have a large lipid core, a thin overlying fibrous cap and an abundance of inflammatory cells. The most important factor determining the plaque stability is the plasma level of atherogenic LDL particles. Increased levels of these particles cause endothelial dysfunction with impaired vasodilatation capacity and prevalence of vasoconstriction, maintain inflammatory infiltration of the plaque, impair the strength of the fibrous cap and facilitate aggregation and coagulation. Effective lowering of plasma cholesterol by pharmacological and non-pharmacological means can revert most of these processes and increase the plaque's mechanical stability within several hours to days. Lipid lowering therapy can therefore decrease the risk of acute coronary events within a very short space of time. Thus a radical decrease in lipid levels, along with modification of other risk factors, may become the cornerstone for treatment of acute coronary syndromes, in addition to being an effective treatment in primary and secondary prevention of coronary heart disease (CHD).

Key words

Cholesterol • Atherosclerosis • Endothelial dysfunction • Acute coronary syndromes • Cholesterol lowering • Plaque stability

Introduction

Acute coronary events (fatal and non-fatal myocardial infarction and sudden cardiac death) remain the leading cause of morbidity and mortality in developed countries, and are therefore in the focus of both basic and clinical research. The last decade brought a significant body of new data that profoundly changed our understanding the pathophysiology of acute coronary events and the possibilities of their clinical treatment. One of the most important findings was the discovery of cholesterol influence on different components of vessel

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wall that has changed our consideration of the role of hypercholesterolemia in the pathogenesis of atherosclerosis. Until recently, our view of the atherosclerotic process was more or less limited to the mechanical aspect of atherosclerotic plaques, creating an obstacle to blood flow. Cholesterol was mainly considered as the major "building brick" of the plaques. Thrombotic occlusion of hemodynamically significant stenoses was presumed to be the most frequent cause of acute coronary events and the regression of already developed plaques was considered the main goal of lipid lowering therapy. Most of our hopes for treatment were based on revascularization techniques, namely percutaneous transluminal coronary angioplasty (PTCA) and coronary artery bypass graft (CABG).

Such a view, however, has recently been questioned from several aspects. The extensive use of revascularization techniques in recent years has not led to the expected decrease in cardiovascular mortality (Jacobs 1999). Studies with lipid lowering drugs showed beneficial clinical effects before it was possible to document regression of plaques on angiography, and such a beneficial effect was also apparent in studies where no regression was documented at all (Waters 1994, Serruys *et al.* 1999, Rabbani and Topol 1999). The groundbreaking observation that plaques most prone to fissure or rupture are often of minor hemodynamic significance, and that most of acute coronary syndromes are caused by stenoses of less than 50 % (Hackett *et al.* 1988) has lead to a major change in our understanding of the pathophysiology of atherosclerosis. The risk of acute artery occlusion therefore does not depend on the hemodynamic significance of the stenosis caused by the plaque, but on its mechanical stability, that makes it prone to fissuring and rupture. The plaques with unfavorable mechanical characteristics are called vulnerable or unstable and are characterized by a high content of lipids, increased activity of inflammatory cells and low mechanical stability (Rabbani and Topol 1999). There is emerging consensus that plaque instability rather than progression should be the most important target for the therapeutic efforts, because its instability underlies most clinical events (Gutstein and Fuster 1999). In fact, in most patients, the first manifestation of the coronary artery disease (CAD) is an acute coronary event, whereas the occurrence of angina on exertion is only found in a minority of patients as the first manifestation (Kannel 1996).

Such clinical observations together with the results of experimental work have gradually changed our view on atherogenesis and the possibilities of influencing it. According to present knowledge, atherogenesis is a dynamic process involving a number of factors: endothelial dysfunction, changes in vessel reactivity, inflammatory infiltration of the subendothelial space, local excess of prothrombotic factors and smooth muscle proliferation with overproduction of the interstitial collagenous matrix. Experimental and clinical reports have shown that the majority of these pathogenic processes are directly influenced by the circulating cholesterol levels and that their activity changes very fast following changes in cholesterol levels (Selwyn *et al.* 1997, Dupuis *et al.* 1999). It is therefore becoming clear that the effects of lowering cholesterol levels exceed the original idea of halting progression or initiating regression of hemodynamically significant stenoses, and that these beneficial effects become apparent immediately after the decrease of cholesterol levels. In this article, we review the most important evidence of cholesterol action on individual components of atherogenesis and list the immediate beneficial effects of lipid lowering therapy together with its mechanisms.

Cholesterol, endothelial function and dynamics of atherosclerotic plaques

The role of endothelium in maintaining normal vessel wall function and endothelial dysfunction

Intact endothelial lining of blood vessels plays a crucial role in maintaining its normal function. The endothelium plays a part in regulating the tension of vascular smooth muscle cells (VSMC) and therefore in blood flow control (Furchgott 1983, Manukhina *et al.* 2000) creates non-thrombogenic inner surface of the vessel, preventing platelet adhesion and coagulation cascade activation, regulates the permeability of the vessel wall for cellular and non-cellular blood components and participates in vessel repair processes and angiogenesis. The endothelium is equipped with receptors for locally and systemically acting mediators and is therefore able to modulate vessel reactivity according to the needs of neighboring tissues in various physiological and pathological processes (Born and Schwarz 1997). The effector arm of endothelium-dependent regulation is mediated by a number of tissue mediators and cytokines. The pivotal role among them

belongs to nitric oxide (NO, EDRF – endothelium-derived relaxing factor). NO is the main mediator causing dilation of vessel wall (Palmer *et al.* 1987). It suppresses expression of cytoadhesive molecules on the surface of endothelial cells, thus limiting the activity of inflammatory cells of monocyte-macrophage lineage (Marui *et al.* 1993), lowers endothelial permeability and increases its anti-aggregation potential and suppresses proliferation and migration of VSMC (Myers and Tanner 1998). NO in the endothelial cells is produced by constitutive NO-synthase (c-NOS), whose production is in turn regulated by numerous factors on the transcription, translation and post-translation level (Wever *et al.* 1998, Feron 1999).

Endothelial lining also mediates the reaction of the vessel wall to mechanical insults as well as other harmful factors and stresses. Endothelium chronically stressed by different influences develops so-called endothelial dysfunction – a uniform reaction the course of which is relatively independent of the precipitating mechanism. It is characterized by limited vasodilatation, excess vasoconstriction, disturbances in coagulation balance and increased permeability of the endothelial lining (McGorisk and Treasure 1996). One of the first consequences of impaired endothelium function that mediates the reactions mentioned above, is the decreased activity of c-NOS (Feron 1999). Endothelial dysfunction is caused by numerous factors, above all by risk factors of atherosclerosis, namely smoking (Lekakis *et al.* 1997), hypertension (Rizzoni *et al.* 1998), hyperglycemia and advanced glycosylation end-products (Chowienczyk and Watts 1997), hyperhomocysteinemia (Doshi *et al.* 1999), and most notably by LDL particles (Creager *et al.* 1990, Galle *et al.* 1998). Endothelial damage can also be caused by mechanical insult (angioplasty, trauma), immune complexes, infections or blood flow turbulence (Born and Schwarz 1997). Endothelial dysfunction is currently thought to be the first stage of atherosclerosis, detectable much earlier than morphological lesions (fatty streaks).

Modified LDL particles, endothelial dysfunction and atherogenesis

LDL particles that had been chemically modified by oxidation or glycosylation play a crucial role in endothelial dysfunction development and its further progression to atherosclerotic lesions (Galle *et al.* 1998). Native LDL particles, however, do not exhibit a significant influence on the function of endothelium and other parts of the vessel wall. Oxidation of LDL particles takes place partially in the plasma but a larger proportion is oxidized after they reach the subendothelial space (Cox and Cohen 1996). Oxidized LDL particles appear in this space even in physiological situations, albeit to a much smaller degree. Normally, their oxidation is not marked and the few oxidized LDL particles are effectively removed by macrophages of the vessel wall *via* their scavenger receptor (Kurihara *et al.* 1991). When the production of oxidized LDL particles is further enhanced, the capacity of macrophages to process them is exceeded and the unprocessed particles cause endothelium damage, inflammatory infiltration of the subendothelial space, VSMC proliferation and overproduction of extracellular matrix (Berliner *et al.* 1990). In the absence of other aggravating factors that would damage the endothelium, overproduction of oxidized LDL particles only occurs in significant hyperlipidemia. However, if the endothelium is damaged for other reasons, i.e. diabetes, its permeability rises and lower levels of circulating lipids are sufficient to lead to accumulation of oxidized LDL particles (Galle *et al.* 1998).

Apart from their direct toxic effects on the endothelium, oxidized LDL particles also cause tissue macrophage dysfunction (Brown and Goldstein 1983). Scavenging of oxidized LDL particles is not regulated (in contrast to the uptake of native particles undamaged by oxidation) and their excess is accumulated in macrophages (Kurihara *et al.* 1991). Such macrophages with excess of cholesterol cannot migrate back into the circulation. They remain trapped in the subendothelial space and change into so-called foam cells (Aviram *et al.* 1998). Foam cells are the basis of fatty streaks, the first morphological stage of atherosclerosis (Stary *et al.* 1994). Apart from their mechanical role in the formation of the plaques, the foam cells are also a source of cytokines that attract other monocytes and T-lymphocytes into the subendothelial space (Hansson *et al.* 1989). These inflammatory cells themselves oxidize LDL particles and produce mediators further aggravating endothelial dysfunction and facilitate migration of more LDL particles through the endothelium. When the excess of cholesterol is sustained, the number of foam cells increases, some of them die and create the soft cholesterol-rich core of atherosclerotic plaques. Mediators produced by foam cells together with oxidized LDL particles cause migration of VSMC from the media into the subendothelial space (Newby and George 1993, Bačáková *et al.* 1999), their proliferation and final transformation into secretory-type cells producing extracellular matrix, notably collagen. Transformed

VSMC and the surrounding extracellular matrix therefore create a fibrous cap over the lipid core of the plaque (Newby and Zaltsman 1999).

Modified LDL particles and plaque stability

Clinical manifestations of atherosclerotic plaques vary according to their majority component – fibrous or lipid. Fibrous plaques can lead to serious narrowing of the vessel lumen and cause chronic ischemia (stable angina on exertion, Gutstein and Fuster 1999). Soft plaques with excess of foam cells and extracellular cholesterol and a thin fibrous cap are often hemodynamically less significant but, due to a decreased mechanical stability, are more prone to fissuring or rupture of the plaque surface (Hackett *et al.* 1988). Mechanical features of atherosclerotic plaques are influenced to a large extent by activity of its inflammatory cells. These cells release mediators, which suppress VSMC activity and produce proteolytic enzymes degrading the extracellular matrix and can lead to mechanical instability of the plaque (Newby and Zaltsman 1999).

If the fibrous cap is damaged, thrombogenic subendothelial material is exposed. This leads to thrombus formation, which can, according to circumstances, lead to a different degree of vessel narrowing and eventually to an acute coronary event. However, the resulting ischemia is always complicated by a thrombus in combination with vessel constriction due to endothelial dysfunction. Due to the high risk of such thrombotic complications, these plaques are called vulnerable or unstable (Weissberg *et al.* 1996, Davies 1996). Morphological properties and plaque stability are determined by numerous factors, most notably by plasma cholesterol levels. Sustained hypercholesterolemia maintains a higher level of subendothelial oxidized LDL particles. These particles in turn perpetuate endothelial dysfunction with its tendency to vasoconstriction and thrombogenity and stimulate paracrine activity of foam cells accentuating inflammatory infiltration and mechanical instability. On the other hand, reduction of plasma cholesterol levels decreases oxidized LDL particle production in the subendothelial space. This is shortly followed by a decrease in inflammatory activity, improvement in endothelial function and increased plaque stability (Aikawa *et al.* 1998a, b). In the case of a significant long-term decrease of cholesterol levels, the lipid core becomes smaller and is eventually replaced by fibrous tissue (Rabbani and Topol 1999, Rosenson and Tangney 1998).

Short-term effects of lipid lowering therapy

Vessel reactivity

Excess vasoconstriction and inability to dilate vessels appropriately in response to stimuli usually causing vasodilatation is frequently found in hypercholesterolemia. The loss of ability to dilate is mainly connected with a limited effect of nitric oxide (NO). Increased production of oxygen radicals caused by excess oxidized LDL particles leads to direct NO inactivation (Ohara *et al.* 1993). Under experimental conditions, oxidized LDL particles lower the transcription activity of the NO-synthase gene, destabilize the mRNA for this protein (Liao *et al.* 1995) and impair the signal transduction between endothelial cell surface receptors and NO production by the G_i protein (Shimokawa *et al.* 1991). Increased thrombogenity of the endothelium activates platelets and releases thromboxane with vasoconstrictor activity. Oxidized LDL triggers these processes in a time- and concentration-dependent manner, and many of these are reversible within hours during a single experiment. The clinical correlate of these pathogenic mechanisms corresponds to impaired endothelium-dependent vasodilatation that can be demonstrated for instance by plethysmography, ultrasound or indirectly by positron emission tomography (PET) (Gould 1998).

Both experimental and clinical studies have repeatedly demonstrated that lowering plasma cholesterol by a diet (Harrison *et al.* 1987), lipid-lowering drugs (Dupuis *et al.* 1999) or by LDL apheresis (Tamai *et al.* 1997) improves endothelium-dependent vasodilatation. The time needed before such improvement can be clinically demonstrated probably depends on the extent and time-course of the decrease in cholesterol levels; in case of dietary intervention it can be expected within months, effective lipid lowering drugs will need weeks to show its effect (Dupuis *et al.* 1999), but in case of rapid cholesterol reduction by 60-80 % after LDL apheresis the improvement is detectable practically immediately (Tamai *et al.* 1997). Cessation of cholesterol feeding and return of serum cholesterol to normal values in monkeys resulted in the restoration of endothelial function and disappearance of intimal inflammation within several

months (Harrison *et al.* 1987). Cholesterol lowering by a diet and cholestyramine significantly improved coronary artery endothelium-dependent vasodilatation after 6 months (Leung *et al.* 1993). NO-dependent vasodilatation in forearm arteries of hypercholesterolemic humans was restored after 12 weeks of lipid-lowering therapy; this beneficial effect disappeared within 6 weeks after the medication had been discontinued and hypercholesterolemia was restored (Stroes *et al.* 1995). Therapeutic lowering of serum cholesterol by LDL-apheresis results in improvement of endothelium-dependent dilation on the forearm immediately after the apheresis (Tamai *et al.* 1997). Similarly, the coronary vasodilatation capacity assessed with PET was significantly improved 18-20 h after single LDL-apheresis (Mellwig *et al.* 1998).

Inflammatory processes in vessel wall

Monocytes and macrophages are thought to be the key cellular elements in the development and progression of atherosclerotic lesions. Moreover, inflammation plays an important role in destabilizing the fibrous cap tissue and causing plaque rupture (Newby and Zaltsman 1999). Macrophages activated by binding of oxidized or acetylated LDL particles to scavenger receptors are a source of cytokines that maintain the inflammatory reaction in the subendothelial space; they produce metalloproteinases that degrade the surrounding extracellular matrix and stimulate VSMC proliferation (Aikawa *et al.* 1998a, Galis *et al.* 1995); finally, they directly aggravate endothelial dysfunction, increase the expression of cytoadhesive molecules on the endothelial cell surface and increase endothelium permeability. All these processes are reversible to a large extent by a decrease in circulating cholesterol levels. It suppresses the expression of cytoadhesive molecules expression on the endothelial cell surface and lowers their plasma concentrations (Corsini *et al.* 1998). The plasma levels of ICAM-1 and ELAM-1 decreased by 25-30 % immediately following LDL apheresis and gradually returned to original levels within 5-7 days (Sampietro *et al.* 1997). Increased adhesiveness of monocytes to endothelial cells in hypercholesterolemic rats is diminished by lipid-lowering treatment (Kimura *et al.* 1997). Lipid lowering therapy also leads to decreased accumulation of cholesterol in macrophages and the production of metalloproteinases by these cells; the number of macrophages in the plaques also decreases with treatment. In an experimental hypercholesterolemic rabbit model, the dietary reduction of serum cholesterol resulted in a marked decrease in the atheroma foam cell content and matrix metalloproteinase activity; this was associated with a substantial accumulation of collagen in the intima and an increase in the proportion of mature VSMC (Aikawa *et al.* 1998a, b).

Systemic inflammatory markers during lipid lowering therapy

The contribution of the local inflammatory reaction to atherosclerotic lesion formation initiated the search for increased systemic markers of inflammation in the plasma. A number of epidemiological studies demonstrated a relationship between the level of some of these proteins (CRP and SAA – serum amyloid A) and the risk of acute coronary event development (Kuller *et al.* 1996). Recently, some works have been published examining the influence of lipid lowering therapy on plasma levels of inflammatory markers. In CARE study the relationship of CRP and SAA levels and acute coronary events was demonstrated; the administration of pravastatin lead to a significant decrease in their levels which correlated with the decrease of coronary risk (Ridker *et al.* 1998). A decrease in CRP was also noted after administration of other statins (simvastatin and atorvastatin) (Strandberg *et al.* 1999).

Platelet function and coagulation factors

The processes largely responsible for total occlusion of the vessel lumen and development of tissue ischemia include platelet aggregation and the formation of thrombi at the site of the damaged endothelial cap of an unstable plaque. The size of the thrombus and the rate of its subsequent dissolution depend on the size and thrombogenity of the exposed subendothelial material and the balance of local and systemic pro- and anti-thrombotic mechanisms (Rosenson and Lowe 1998). Platelet aggregability is directly dependent on endothelial function and endothelial dysfunction is accompanied by increased aggregability and thrombogenic potential of platelets (Badimon *et al.* 1991). The levels of platelet cGMP, whose production is dependent on endothelial NO synthesis, are lower at higher plasma cholesterol levels. However, cholesterol also influences the aggregability of platelets directly; patients with hypercholesterolemia have a higher cholesterol:phospholipid ratio in platelet membranes, leading to their increased aggregability

(Mazeaud *et al.* 1992). Lowering cholesterol levels by pharmacological as well as non-pharmacological methods leads to normalization of endothelial function and of the lipid composition of platelet membranes and therefore to a decrease in their aggregability (Hochgraf *et al.* 1994, Rosenson and Tangney 1998). Hypercholesterolemia is also associated with a procoagulant state, although only to a limited extent (Barrowcliffe *et al.* 1985, Rosenson and Lowe 1998). For example, there is stimulation of the plasminogen activator inhibitor (PAI-1), inhibition of the plasminogen activator (Levin *et al.* 1994), induction of the procoagulant tissue factor mRNA, inhibition of mRNA transcription of thrombomodulin and stereochemical alteration in heparan sulfate proteoglycans (Rosenson and Lowe 1998). The majority of these changes are the result of diminished availability of NO (Holvoet and Collen 1995, Cox and Cohen 1996). Based on theoretical assumptions, correction of these abnormalities would be expected with lipid lowering therapy (Lacoste *et al.* 1995). However, the results of clinical observations are conflicting because a decrease (Isaacsohn *et al.* 1994) as well as an increase (Davidson *et al.* 1997) in PAI-1 levels has been noted after administration of statins. Colli *et al.* (1997) demonstrated a decrease in procoagulant tissue factor synthesis by human macrophages. Administration of fibrates leads, apart from the lipid lowering effect, to a decrease in fibrinogen levels, which is probably an effect independent of their lipid-lowering action (Watts and Dimmitt 1999). No significant influence of a low lipid diet on coagulation parameters has been noted. LDL apheresis significantly lowers the levels of fibrinogen, however, this is due to the direct elimination effect of the method, independent of its influence on cholesterol.

Lipid lowering and "non-lipid related effects" of hypolipidemic agents

We have briefly summarized here current information about the immediate effects of lowering cholesterol levels on individual components of the atherosclerotic process. The administration of lipid lowering drugs has become an important tool in lowering plasma cholesterol levels and many of the laboratory and clinical observations have been made using these drugs. After the long-held beliefs on the role of cholesterol in atheroma development, the recent discoveries of short-term effects of the lipid lowering treatment came as a certain surprise. It seemed that such effects cannot be explained by decreased cholesterol levels and this led to the conclusion that they must be a consequence of non-lipid related action of the drugs. These effects are called "non-lipid related", "extralipid" or "pleiotropic" and are most often mentioned in conjunction with statins (Vaughan *et al.* 1996), though they were described in other lipid-lowering drugs.

"Non-lipid related" effects of lipid-lowering drugs are a new and interesting area of research. Their clinical significance, however, is still not clear and somewhat speculative, based on experimental observations rather than on clinically proven effects (Davignon and Laaksonen 1999). Short-term effects of cholesterol lowering were satisfactorily explained theoretically. Experimental results discussed in this review were obtained by different methods of achieving decreased cholesterol levels which confirmed each other. From the clinical point of view, a comparable decrease in the incidence of cardiovascular events after pharmacological and non-pharmacological lowering of cholesterol (ileal bypass, diet, LDL-apheresis) confirms the hypothesis that the basic factor determining the effects of treatment is the actual decrease in cholesterol levels. On the contrary, no study is available to confirm the significant contribution to improved prognosis by "non-lipid related" effects of lipid-lowering drugs. This is further supported by recent meta-analysis of lipid lowering studies, including non-pharmacological ones. The only factor determining the beneficial effect of the treatment was the extent of cholesterol level decrease achieved in individual studies (Gould *et al.* 1997).

Conclusions and perspectives

Recent large intervention studies have shown a significant decrease of cardiovascular morbidity and mortality directly attributable to lipid lowering therapy (Gould *et al.* 1997). The beneficial clinical effect of such therapy was apparent earlier than expected; in angiographic studies the improved prognosis was noted before it was possible to document the regression of developed atherosclerotic lesions and was present even in patients without any documented regression of stenoses (Waters 1994, Serruys *et al.* 1999, Rabbani and Topol 1999). The results of recent research provided new data explaining these early beneficial effects of lipid lowering therapy.

The morphological basis of a majority of serious cardiovascular events is the rupture of vulnerable

atherosclerotic plaques with subsequent thrombosis and occlusion of the affected vessel. All developmental stages of a vulnerable plaque are directly influenced by atherogenic lipoproteins: modified LDL particles induce endothelial dysfunction, represent the basis for foam cells development, perpetuate the inflammatory reaction within the plaque and therefore decrease its elasticity. In the case of plaque rupture they facilitate aggregation and coagulation of blood and increase the risk of thrombotic vessel occlusion. Laboratory as well as clinical observations have confirmed that lowering cholesterol levels gradually normalizes all these pathological processes and that some of the beneficial effects become apparent shortly after a significant reduction of cholesterol levels was achieved. Contrary to the previous beliefs that the benefits of lipid lowering therapy can only be seen several years later, it is obvious now that the decrease of cardiovascular risk can be demonstrated within less than a year, and that impaired vessel reactivity normalizes within weeks. If a significant reduction in circulating cholesterol is achieved, it is even possible to demonstrate a decrease in endothelial dysfunction within several hours (Tamai *et al.* 1997, Sampietro *et al.* 1997).

These data have been procured in treating patients with chronic forms of ischemic heart disease or in patients with hyperlipidemia without clinically overt atherosclerosis. They have opened up new routes for lipid lowering therapy – intervention in acute ischemic syndromes in the coronary, cerebral and peripheral circulation. Cholesterol is the major factor jeopardizing the stability of the plaque, and reduction of plasma cholesterol influences it almost immediately. It is therefore prudent to assume that such therapy can improve the clinical manifestations of acute ischemia. The initial studies assessed the effect of lipid lowering therapy in acute myocardial infarction, and the results of the first large mortality study will be published shortly (Schwartz *et al.* 1998). The beneficial effect of a transient dramatic decrease in plasma cholesterol after LDL apheresis has been demonstrated in patients with different forms of acute cerebrovascular ischemia (Suckfull *et al.* 1999).

The past decade has thus changed entirely our understanding of the possibilities of lipid lowering therapy in primary as well as secondary prevention of ischemic heart disease and other clinical manifestations of atherosclerosis. The results of basic research have contributed to this change by describing the pathophysiological mechanisms of the atherothrombotic process that results from interaction of atherogenic lipids and other risk factors, vessel wall and blood constituents. This interaction is dynamic and can be modified within a relatively short time. More experimental data are required to refine our current strategies targeted to vessel wall dysfunction in acute ischemic syndromes. Radical lipid lowering with modifications of other risk factors may become the cornerstone for the treatment of acute coronary syndromes, in addition to being an effective approach to primary and secondary prevention of coronary heart disease.

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