

## Endothelial dysfunction – A predictor of atherosclerosis

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**ABSTRACT:** Endothelial dysfunction is a systemic disorder and a critical element in the pathogenesis of atherosclerotic diseases and its complications. Growing evidences suggest that the individual burden of currently known cardiovascular risk factors is not the only determinant of endothelial function; rather endothelial integrity depends on the balance of all cardiovascular risk factors and vasculoprotective elements in a given individual, including the genetic predisposition. Recent studies have demonstrated that patients with endothelial dysfunction have an increased risk of clinical cardiovascular events. The coronary events including myocardial infarction, death or neovascularization occur only in patients with severely impaired endothelial functions. Interventions like risk factor modification and treatment with various drugs may improve prognosis. Hence given its reversibility and granted the availability of a diagnostic tool to identify patients at risk and to control the efficacy of therapy in clinical practice, endothelial dysfunction may be an attractive target, in an effort to optimize individualized strategies to reduce cardiovascular morbidity and mortality. Thus a deep insight in to the pathophysiology of endothelium and its functions can be of value in identifying and preventing the risk factors of various cardio vascular diseases. The early risk prediction would ensure a better quality of life in the adulthood if the preventive steps are taken in the childhood itself since there is no age bar for the onset of the endothelial dysfunction.

**KEY WORDS:** Endothelial dysfunction; Atherosclerosis; Cardiovascular events; Risk factor; Prognosis

### INTRODUCTION

Endothelium is an inert lining of the blood vessels, but is highly specialized, metabolically active interface between blood and the underlying tissues. The endothelium plays a vital role in vascular homeostasis, vascular tone regulation, vascular smooth cell proliferation, trans endothelial leukocyte migration, thrombosis and thrombolytic balance. In response to various mechanical and chemical stimuli, endothelial cells synthesize and release a large number of vasoactive substances, growth modulators and other factors that mediate these functions.

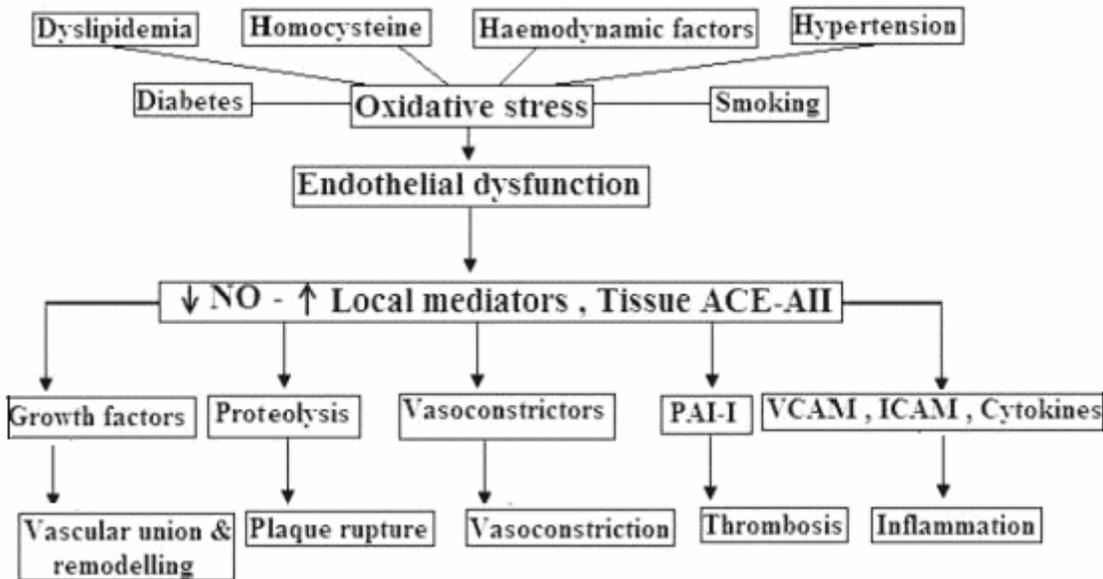
Endothelial dysfunction can be defined as, “the partial or complete loss of balance between vasoconstrictors and vasodilators, growth promoting and growth inhibiting factors, proatherogenic and anti-atherogenic factors”<sup>1</sup>. Endothelial dysfunction is now regarded as an early pivotal event in atherogenesis<sup>2</sup> and has been shown to precede development of clinically detectable atherosclerotic plaques in the coronary arteries<sup>3</sup>. It has also been considered an important event in the development of micro vascular complications in diabetes<sup>4</sup>.

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In endothelial dysfunction, there is reduction in the bio-availability of vasodilators, in particular, nitric oxide (NO), whereas endothelial derived contracting factors are increased<sup>5</sup>. On the other hand, endothelial dysfunction, aside from denoting impaired endothelium dependent vasodilatation, also comprises a specific state of 'Endothelial activation' which is characterized by a pro-inflammatory, proliferative and

procoagulatory milieu that favours all stages of atherogenesis (Figure 1)<sup>6</sup>. Given this relationship between endothelial dysfunction and atherosclerosis, it is likely that the status of endothelial function may reflect the propensity of an individual to develop atherosclerotic disease, and thus the presence of endothelial dysfunction may serve as a marker of an unfavorable cardiovascular prognosis<sup>7</sup>.



**Figure 1:** Shows the central role of endothelial function in the causation and progression of atherosclerosis. Endothelial dysfunction not only denotes impaired endothelial dependent vasodilatation, but also depicts a state of endothelial activation which is characterized by a proinflammatory, proliferative and procoagulatory milieu that favours all stages of atherogenesis.

**ENDOTHELIAL DYSFUNCTION AND ATHEROSCLEROSIS**

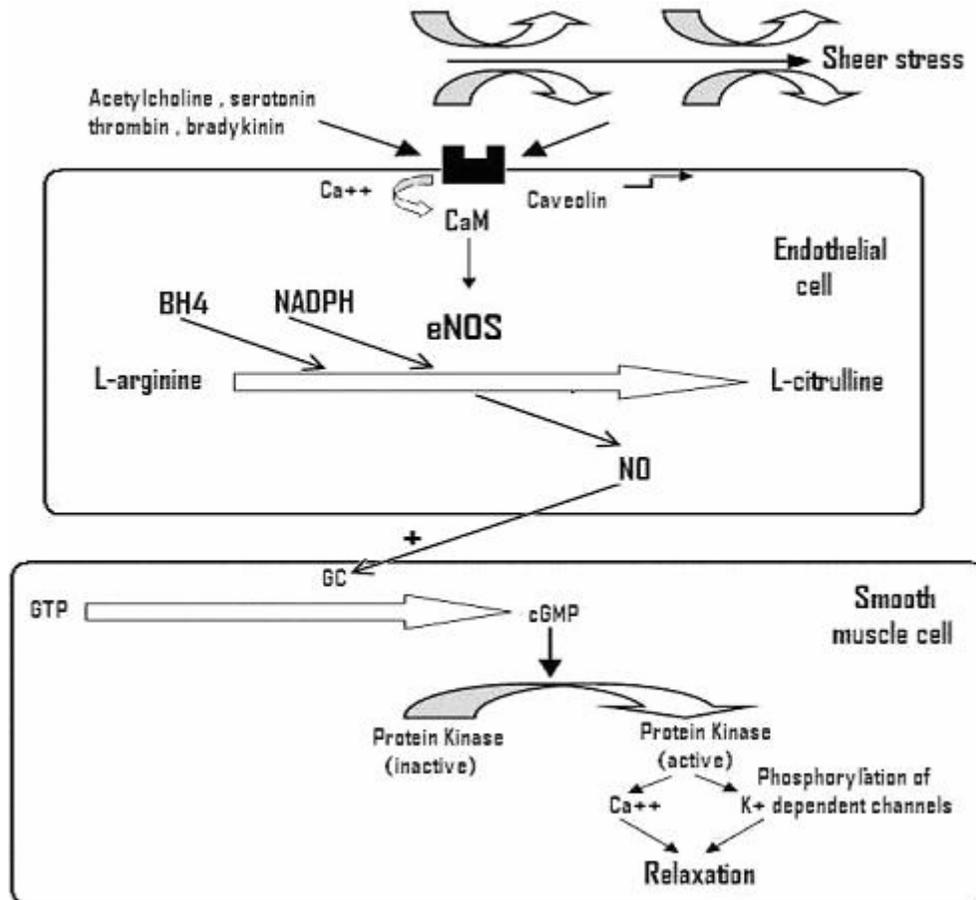
Recent insight into the basic mechanisms involved in atherogenesis indicate that deleterious alterations of endothelial physiology, also referred to as endothelial dysfunction, represents a key early step in the development of atherosclerosis and are also involved in plaque progression and the occurrence of atherosclerotic complications<sup>8</sup>.

Although the association between cardiovascular risk factors and atherosclerotic disease is well documented, the mechanism by which these risk factors induce lesion formation and lead to events is not entirely defined. Given its strategic location and biological properties, the endothelial cell layer that represents a mechanical and biological barrier between the

blood and the vascular wall and is likely to serve as the 'missing link' between any given risk factor and its detrimental vascular effects<sup>9</sup>. Most, if not all risk factors that are related to atherosclerosis and cardiovascular morbidity and mortality, including traditional and nontraditional risk factors, are also found to be associated with endothelial dysfunction. Many of these risk factors, including hyperlipidemia, hypertension, diabetes and smoking are associated with overproduction of reactive oxygen species or increased oxidative stress<sup>10</sup> (Figure 2). By reacting with NO, ROS may reduce vascular NO bioavailability and promote cell damage. Hence increased oxidative stress is considered a major mechanism involved in the pathogenesis of endothelial dysfunction and may serve as a common pathogenic mechanism of the

effect of risk factors on the endothelium<sup>11-13</sup>. Risk to develop endothelial dysfunction increases with the number of risk factors present in an individual but the potential to alter endothelial function may vary between risk factors<sup>14</sup>. Taken together, the status of endothelial function represents an integrated index of both the overall cardiovascular risk factor burden and the sum of all

vasculoprotective factors in any given individual<sup>15</sup>. Moreover, given its pivotal role in the atherogenic process, endothelial dysfunction may be regarded as the “ultimate risk of the risk factors” indicating the existence of a specific atherogenic vascular milieu<sup>7</sup> which is associated with perfusion abnormalities and cardiovascular events.



**Figure 2: Production of nitric oxide (NO) by endothelial cells. NO is produced by the action of endothelial nitric oxide Synthase (eNOS) on L-arginine. This reaction requires a number of cofactors, including tetrahydrobiopterin (BH<sub>4</sub>) and nicotinamide adenine dinucleotide phosphate (NADPH). Increased intercellular Ca<sup>++</sup> in response to vasodilator agonists or shear stress displaces the inhibitor caveolin from calmodulin (CaM), activating eNOS. NO diffuses to vascular smooth muscle and causes activation of protein kinase which causes phosphorylation of k<sup>+</sup> dependent channels resulting in relaxation of the smooth muscles.**

**SUBSTANCES RELEASED BY THE ENDOTHELIUM**

Endothelium releases a group of substances to maintain the vascular homeostasis. Nitric oxide, Bradykinin, Prostacyclin, Serotonin, Histamin,

Substance P and Endothelium- derived hyperpolarizing factor are the vasodilators while the substances like Angiotensin, Endothelin (ET-I), Thromboxane A<sub>2</sub>, Serotonin, Arachidonic acid, Prostaglandin H<sub>2</sub> and Thrombin are the

vasoconstrictor molecules released by the endothelium<sup>16</sup>. Platelet derived mediators, such as Serotonin, induce vasoconstriction in the presence of a dysfunctional endothelium. Moreover, Vasoconstrictor response to these stimuli is increased by Endothelin-1<sup>17</sup>, concentration of which are elevated in the plasma of patients with early and advanced atherosclerosis<sup>18</sup> as well as in culprit lesions.

A healthy endothelium is characterized by an antithrombotic milieu mediated by the secretion of various factors that exert antiaggregatory effects on platelets (NO and prostacyclin) or

have anticoagulatory (heparin and protein/s) or fibrinolytic effects (by tissue plasminogen activator, plasminogen activator inhibitor-1 and thrombomodulin).

Endothelium derived NO, Prostacyclins, Bradykinin, Heparin sulfate, transforming growth factor, etc are the inhibitors of the smooth cell growth which tend to balance the effect of the smooth cell growth promoters which are Platelet derived growth factors, Basic fibroblast growth factor, Insulin like growth factor-1, Endothelin and Angiotensin etc. (**Table 1**)

**Table 1: Substances released by endothelium**

Substances	Examples
<i>Vasodilators</i>	Nitric oxide; Bradykinin; Prostacyclin; Endothelium-derived hyperpolarizing factor; Serotonin; Histamine; Substance P
<i>Vasoconstrictors</i>	Angiotensin II (AII); Endothelin (ET-1); Thromboxane A2; Serotonin; Arachidonic acid; Prostaglandin H2; Thrombin
<i>Promoters</i>	Platelet derived growth factor (PDGF); Basic fibroblast growth factor (PGF); Insulin-like growth factor – I (IGF-I); Endothelin (ET1); Angiotensin
<i>Inhibitors</i>	Nitric oxide; Prostacyclin; Bradykinin; Heparin sulfate; Transforming growth factor I (TGFB)
<i>Adhesion molecules</i>	Endothelial leukocyte adhesion molecule; Intercellular adhesion molecule (ICAM); Vascular cell adhesion molecule (VCAM)
<i>Thrombolytic factors</i>	Tissue-type plasminogen activator; Plasminogen activator inhibitor-1 (PAI-I); Thrombomodulin

### ROLE OF NITRIC OXIDE

The most critical of the substances released by endothelium is nitric oxide (NO) (**Table- 1**) which is produced by the conversion of the substrate L-Arginine to L-Citrulline by the enzyme, Nitric oxide Synthase (eNOS) (**Figure 2**). NOS is activated, upon stimulation of endothelium by a variety of stimuli including shear stress, substance P and Acetyl choline<sup>8</sup>. NO thus formed diffuses into the underlying vascular smooth muscles to stimulate the activation of Guanylate cyclase, which in turn converts GTP to the smooth muscle relaxant compound cGMP in the vascular smooth muscle cells<sup>19</sup>.

The increase in cGMP leads to activation of cyclic GMP- dependent protein kinase, which in turn leads to phosphorylation of potassium dependent channels with consecutive hyper polarization and extrusion of calcium ions resulting in VSMC (Vascular Smooth Muscle Cell) relaxation<sup>20</sup>.

NO not only produces vasodilatation, but it also participates in various processes that are beneficial to the vasculature such as reduction of

vascular smooth muscle cell migration and growth, platelet aggregation and thrombosis, monocyte and macrophage adhesion and inflammation (**Table1**)<sup>21</sup>. In general the substances released by endothelium have important autocrine as well as paracrine functions and help to maintain not only the normal health of vascular wall but also control the haemostatic functions and the hemodynamic balance of the entire body<sup>22</sup>.

### ENDOTHELIAL DYSFUNCTION AS CLINICAL SYNDROME

The presence of endothelial dysfunction can be regarded as a clinical syndrome that per se is associated with and predicts an increased rate of adverse cardiovascular events<sup>21</sup>.

**Endothelial dysfunction and acute coronary syndrome:** Endothelial dysfunction may play a fundamental role in the pathogenesis of acute coronary syndromes (ACSs); such as unstable angina and acute myocardial infarction<sup>22</sup>. Endothelial dysfunctions contributes to enhanced plaque vulnerability owing to reduced anti-inflammatory potential, and favours thrombus

formation due to reduction in the anticoagulatory potential and increase in endothelial production of procoagulatory mediators (e.g. tissue factor and plasminogen activator inhibitor) and thus may be viewed as an important contributory factor for several aspects of ACSs<sup>23</sup>. Various studies have provided evidence for a direct contribution of coronary endothelial dysfunction to myocardial perfusion defects associated with intracoronary administration of acetylcholine in patients without obstructive CAD<sup>7</sup>.

**Endothelial dysfunction and hypertension:** Hypertension is directly implicated in the pathophysiology of various cardiovascular disease states and is a significant contributor to ill health, leading to an excess of both morbidity and mortality. The etiology of hypertension has been explored in depth, but the pathophysiology is multifactorial, complex, and poorly understood. Recent interest has been directed toward investigating the purported role of the endothelium, which acts as an important regulator of vascular homeostasis. Endothelial dysfunction is now recognized to occur in hypertension, regardless of whether the etiology is essential or secondary to endocrine or renal processes<sup>24</sup>. The pathogenesis of systemic sclerosis (SSc) includes vasculopathy with endothelial dysfunction<sup>25</sup>.

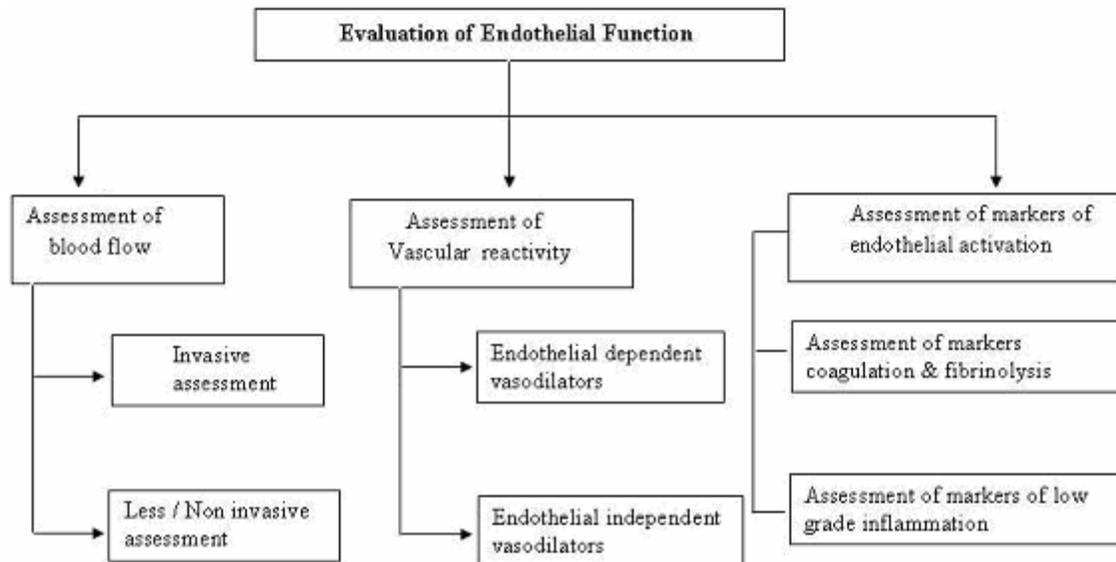
Nitric oxide (NO) acts to maintain vascular tone. Reduced bioavailability of NO appears to be the key process through which endothelial dysfunction is manifested in hypertension. The result is of an imbalance of counteracting mechanisms, normally designed to maintain vascular homeostasis, leading to vasoconstriction and impaired vascular function. It has become increasingly apparent that these changes may be effected in response to enhanced oxidative stress, possibly as a result of systemic and localized inflammatory responses<sup>24</sup>.

#### **EVALUATION OF ENDOTHELIAL FUNCTION**

There is no single and standard test to fully assess the endothelial functions in vivo. Because the endothelium regulates several vascular functions, the assessment of each of them is a potential way to evaluate its integrity. A common approach to the evaluation of

endothelial function is the assessment of blood flow and vascular reactivity since the first description of endothelial dysfunction in atherosclerotic epicardial coronary arteries in 1986 by **Ludmer** and colleagues<sup>26</sup>. Invasive assessment of coronary endothelial function by quantitative coronary angiography and coronary Doppler flow measurements, along with graded intracoronary infusions of endothelium dependent vasodilators such as acetylcholine were considered the 'Gold standard' for endothelial function testing<sup>27</sup>. However, during the last decade, other less invasive or noninvasive techniques for the assessment of endothelial function including strain gauge forearm plethysmography in conjunction with intra-arterial infusion of endothelium dependent vasodilators such as methacholine or acetylcholine and high resolution external vascular ultrasound to measure flow mediated endothelium dependent dilatation (FMD) of the brachial artery during reactive hyperemia, have been developed. These techniques are based on the fact that endothelial dysfunction is not confined to the coronary arteries but rather represents a systemic disorder that also affects vascular beds including both conduit arteries and small resistance vessels in the extremities<sup>28</sup>.

Another approach is to measure levels of the members of endothelial activation, such as soluble vascular cell adhesion molecule (VCAM), soluble intracellular adhesion molecules (ICAM), Endothelin-I (ET-I) and other markers of coagulation and fibrinolysis such as PAI-I, Tissue plasminogen activator or Von-Willebrand factors (VWP) and markers of low grade inflammation such as C-reactive proteins, IL-1, IL-6, and TNF-alpha.<sup>29</sup> The identification and quantification of circulating endothelial cells (CECs) has evolved as a novel marker of endothelial function. As a technique, it correlates with other markers of endothelial function such as flow-mediated dilation, the measurement of von Willebrand factor, and tissue plasminogen activator. Quantification of CECs is difficult due to low numbers, variable morphology, and a lack of standardization in current techniques used. CECs appear to be a different population of cells to endothelial progenitor cells<sup>30</sup>. **(Figure 3)**



**Figure 3: Shows the approach to evaluation of endothelial function. It is the assessment of blood flow and vascular reactivity. The stimulation of the endothelium to produce nitric oxide is used to assess endothelial-dependent vasodilatation whereas the stimulation of VSMCs is used to assess endothelium – independent vasodilatation**

### THERAPEUTIC STRATEGIES TO IMPROVE ENDOTHELIAL FUNCTION

Endothelial dysfunction is a reversible disorder, and strategies aimed at reducing cardiovascular risk factors, such as cholesterol lowering, antihypertensive therapy, smoking cessation, estrogen replacement therapy in postmenopausal women, supplementation with folic acid and physical exercise also translate into an improvement in endothelial health.

The nonpharmacological approaches like life style modifications and decrease oxidative stress improve insulin sensitivity and correct dyslipidemia and thus improve endothelial function<sup>31</sup>.

A number of pharmacological interventions have been shown to improve endothelial dysfunction. Since dyslipidemia is frequently associated with endothelial dysfunction. The Endothelial Protective effects may be mediated by the 'Statin's, antioxidant, anti-inflammatory properties and their capability to restore vascular NO bio-availability.

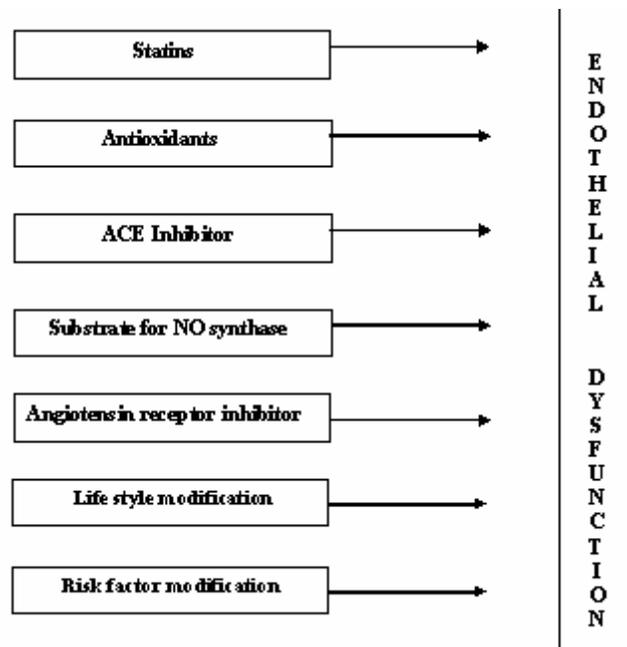
Angiotensin converting enzyme inhibitors (ACEIs) increase NO bio-availability by decreasing the synthesis of Angiotensin II and by enhancing serum levels of NO-releasing Bradykinin via inhibition of its degradation. Moreover ACEIs may also enhance the activity of endothelium derived hyperpolarizing factors under certain conditions<sup>32</sup>. The result of TREND

study showed that the treatment with Quinapril was associated with significant improvement in endothelial dependent vasodilatation in CAD patients with evidence of endothelial dysfunction at baseline<sup>33</sup>. The long term clinical benefits of treatment with ACE inhibitors with high affinity for tissue ACE activity were recently documented by the results of the HOPE study, which showed highly significant fall in all cardiovascular events in patients treated with Ramipril<sup>34</sup>.

In contrast to ACEIs, controversial results have been reported regarding the effects of Angiotensin receptor antagonists, which specifically block the effects of Angiotensin II on the Angiotensin type I (AT-I) receptors, on endothelial function<sup>35</sup>.

Given that increased oxidative stress plays a pivotal role in the pathogenesis of endothelial dysfunction, administration of antioxidants would be expected to be a reasonable strategy to treat this disorder. Supplementation with antioxidants such as Glutathione, N-acetyl cysteine, and vitamin C has been shown to reverse endothelial dysfunction in coronary and peripheral arteries<sup>36</sup>.

Supplementations with substrate for NO synthesis i.e. arginine and calcium channel blockage with Nifedipine also improve endothelial function<sup>33</sup>. **(Figure 4)**



**Figure 4: Shows the therapeutic strategy for treating the endothelial dysfunction and thus modifying the Ultimate risk factors of CAD**

**CONCLUSION**

Endothelial dysfunction is a systemic disorder and critical element in the pathogenesis of atherosclerotic diseases and its complications. Growing evidences suggest that the individual burden of currently known cardiovascular risk factors is not the only determinant of endothelial function; rather endothelial integrity depends on the balance of all cardiovascular risk factors and vasculoprotective elements in a given individual, including the genetic predisposition. Given the important role of endothelial dysfunction for the development and progression of atherosclerosis, aside from the treatment of established cardiovascular risk factors, endothelial dysfunction seems a primary therapeutic target in the prevention of atherosclerotic disease. Such a therapeutic strategy would require the appropriate diagnostic tool that allows the identification of candidate patients. Non-invasive, easy to perform, accurate, reproducible and inexpensive techniques are required to evaluate endothelial function / dysfunction. Thus to date, targeting the established and modifiable cardiovascular risk factors remain the primary strategy to improve endothelial function and prognosis in individual at risk for atherosclerotic disease. However, as techniques to reliably assess endothelial function will become available, targeting endothelial dysfunction to

optimize individualized risk reduction strategies might become a reality in future.

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