

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

I. THE ENDOTHELIUM AND ITS FUNCTIONS

The vascular endothelium performs an array of homeostatic functions within the normal blood vessels.

Located between the vessel lumen and the vascular smooth muscle cells, the endothelium is a monolayer of cells capable of transducing blood-borne signals, sensing mechanical factors within the lumen, and regulating vascular tone through the production of a variety of vasoactive humoral factors.

Functions: –

- 1) Vasodilatation or vasoconstriction to regulate blood flow.
- 2) Growth or differentiation of vascular smooth muscle cells.
- 3) Anti-inflammatory or pro-inflammatory effects.
- 4) Maintenance of normal fluidity of blood and avoidance of bleeding.

Autocrine and Paracrine Substances released by the endothelium. (6)

Type of Substance	Specific Substances
Vasodilators	NO, prostacyclin, bradykinin, endothelium-derived hyperpolarizing factor, C-natriuretic peptide.
Vasoconstrictors.	ET-1, Angiotensin -II, Thrombox. A ₂ , Oxidant radicals, PG H ₂ .
Antiproliferative	NO, prostacyclin, TGF- β .
Proproliferative	ET-1, Angiotensin -II, oxidants, platelet-derived growth factor, Insulin-like growth factor, interleukins.
Antithrombotic	NO, prostacyclin, protein-C, plasminogen activator, von willebrand factor.
Prothrombotic	ET-1, oxidant radical, T _x A ₂ , Plasminogen, activator inhibitor – 1. fibrinogen, tissue factor.
Inflammatory markers	Cellular adhesion molecules. ICAM, VCAM, P and E – Selection)
Angiogenesis	Vascular endothelial growth factor.

Normally, the endothelium promotes vasodilatory functions in response to a variety of systemic, neurohumoral and mechanical stimuli. Inappropriate vasoconstriction characterizes the vascular response in patients with endothelial dysfunction. (6)

An imbalance among the endothelial-derived counteracting vasoactive factors occurs in vascular segments damaged early in the atherosclerotic process.

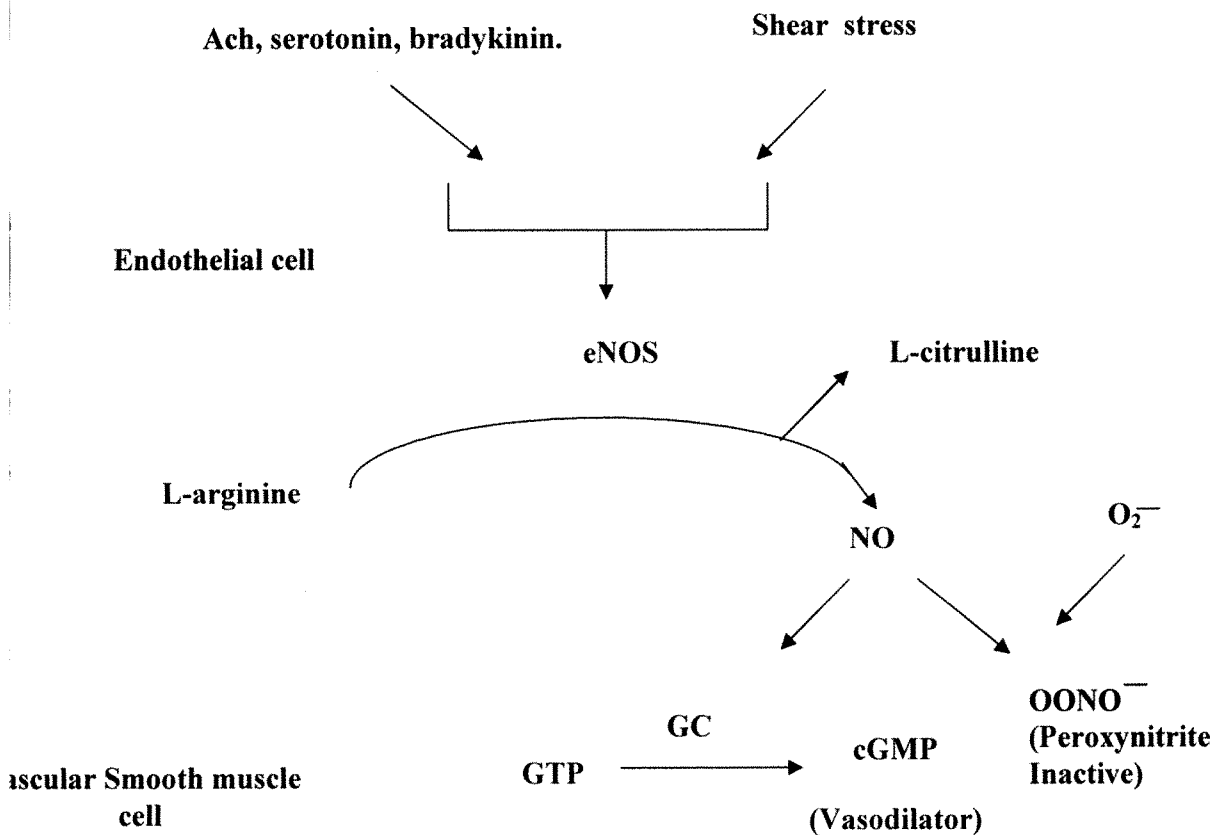
Dysfunctional endothelium, common in patients with cardiovascular risk factors, leads to disturbance in coronary blood flow, promoting myocardial ischaemia, and accelerating the evolution of atherosclerosis and thrombosis. (7)

ENDOTHELIUM DERIVED RELAXING FACTORS

NITRIC OXIDE - (NO)

NO is one of the key mediators of the endothelium cell function. It is formed inside the endothelial cell from L-arginine by the action of NO synthase. (eNOS) (8)

NO diffuses into the smooth muscle cells activating intracellular guanylate cyclase, increasing cGMP and consequently reducing intracellular calcium.. This leads to vasodilatation. (9)



Once released from endothelial cells, NO has a very short half life due to interaction with other free radicals (Superoxide).

The release of NO is stimulated by products of thrombosis (Thrombin), aggregating platelet (serotonin, ADP) and other chemical stimuli (histamine, bradykinin), and by shear stress with flow-mediated vasodilatation.

In contrast to NO, the nitro-vasodilators (Nitroglycerine, nitropursside) act independently of the endothelium and directly on vascular smooth muscle. NO also inhibits the recruitment and differentiation of inflammatory cells by inhibiting the production of chemo attractant cytokines, leukocyte adhesion molecules, and factors involved in differentiation of monocytes into macrophages.

Reduction in NO are associated with activation of potentially vulnerable atherosclerotic plaques and acute coronary syndromes. (10)

ENDOTHELIUM-DERIVED HYPERPOLARIZING FACTOR (EDHF)-

Endothelium dependant vasodilatation also occurs by hyperpolarizing the underlying smooth muscle through activation of Ca^{2+} activated K^{+} channels, by a factor termed EDHF (11,12-epoxyeicosatrienoic acid)

It appears to be more important in smaller arterioles than in larger conduit arteries. (12)

ENDOTHELIUM-DERIVED CONSTRICTING FACTORS -

The endothelium is also a source of vasoconstrictor factors such as the endothelin and Angiotensin – II.

ENDOTHELIN 1 (ET-1).

This is a 21 Amino acid peptide that has a potent vasoconstrictor activity.

Unlike NO, which can be released in response to stimuli and then inactivated within seconds, ET-1 mediated vasoconstriction is slow in onset and lasts over minutes to hours.

ET-1 also stimulates smooth muscle proliferation, vascular remodeling, and leukocyte adhesion and recruitment. (13)

ET -1 exerts its effects by binding to two specific receptors named ET-A and ET-B. Plasma concentration of ET-1 is elevated in a number of cardiovascular disorders including hypercholesterolemia, hypertension, acute myocardial infarction and congestive heart failure. (13)

Angiotensin – II

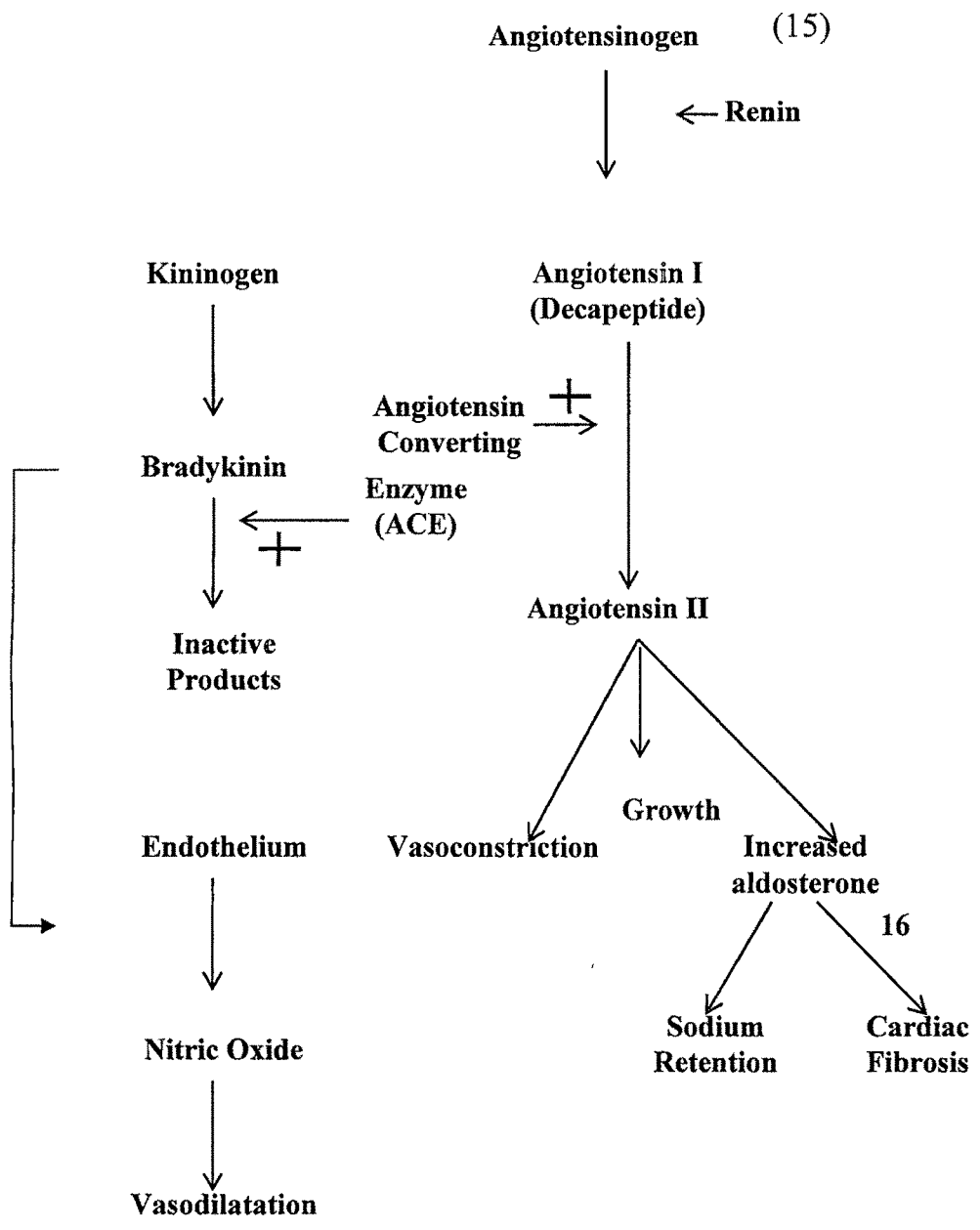
Angiotensin – 1 originates in the liver from angiotensinogen under the influence of the enzyme renin, a protease, that is formed in the renal juxtaglomerular cells.

The Angiotensin Converting Enzyme (ACE) converts angiotensin I to angiotensin –II(an octapeptide), and also inactivates bradykinin.

Angiotensin – II exerts its effect upon several vascular smooth muscle cell functions like constriction, growth, proliferation, and differentiation. Overall, the actions of angiotensin – II oppose those of nitric oxide (14)

BRADYKININ SYSTEM

ANGIOTENSIN SYSTEM



In addition to its effects on Angiotensin – I, ACE also acts to convert bradykinin into inactive products.

Thus, bradykinin induced Nitric Oxide synthesis reduces and this also contributes to endothelium dysfunction.

INFLAMMATORY MEDIATORS

The endothelial cell is also involved in the production of adhesion molecules such as intracellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), endothelial laminar adhesion molecule (ELAM). P- and E-selectin, and chemokines.

These molecules serve to attract and anchor cells involved in the inflammatory reaction. It has been demonstrated that the atherosclerotic process is associated with an increased blood level of acute phase proteins and hence inflammation (17).

II HYPERTENSION AND VASCULAR DISEASE

Approximately 1 billion individuals in the world are affected by hypertension.

About 7 million deaths are attributable to hypertension.

sub-optimal blood pressure control is the number one risk factor for death throughout the world.

Hypertension, if untreated is associated with a shortening of life by 10 to 20 yrs due to acceleration of the atherosclerotic process. (18)

For every 20 mm Hg systolic or 10 mm Hg diastolic increase in the blood pressure, there is doubling of mortality from both stroke and ischaemic heart disease.

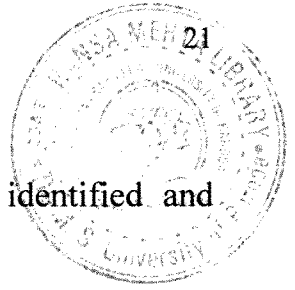
The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure (2003) i.e. JNC -7 classifies hypertension as follows

BLOOD PRESSURE (MMHG)

-	<120/80	Normal
-	120-139/80-89	Prehypertension.
-	$\geq 140/90$	Hypertension.
	(140 – 159/90-99	Stage 1
	$\geq 160/100$)	Stage 2.

The JNC 7 report has introduced the term “prehypertension” for those with BP ranging from 120-139 mmHg systolic and/or 80-89 mmHg diastolic.(19) This new designation is intended to identify individuals in whom early intervention by adoption of healthy lifestyles could reduce BP, or decrease the rate of its progression.

Another change from the JNC-6 is the combining of stage 2 and stage 3 hypertension into a single stage 2 category. This reflects that the management of the former two groups is similar .



A number of causal factors for hypertension have been identified and include (20)

- Excess body weight (obesity)
- Excess dietary sodium intake.
- Reduced physical activity
- Insulin resistance.
- Excess alcohol intake.

Hypertension is closely associated with atherosclerosis.

The Framingham study and the Multiple Risk Factor Intervention Trial (MRFIT) have demonstrated that moderate to severe hypertension significantly increase the atherogenesis.

The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study also showed that blood pressure is associated with acceleration of atherosclerosis, especially fibrous plaque development.

Hypertension can lead to a number of complications including –

- Effects on the heart -
 - Left ventricular hypertrophy
 - Congestive heart failure
 - Coronary artery disease
- Effect on CNS
 - Hypertensive retinopathy
 - Cerebrovascular stroke – cerebral infarction
 - cerebral hemorrhage.
 - Hypertensive cephalopathy
- Effect on kidneys
 - Arteriosclerosis of afferent and efferent vessels
 - Chronic renal failure.

Many of these effects are caused by an acceleration of the atherosclerotic process in hypertensive subjects. (21)

As endothelial cell dysfunction is an early event in atherosclerosis, detection of endothelial dysfunction could be valuable in predicting these complications.

It has been show that endothelial dysfunction in hypertension is independent from the etiology of hypertension or from vascular structure. (24)

2) Diabetes Mellitus

The cellular and molecular basis for endothelial cell dysfunction is as follows

- Hyperglycemia leads to activation of protein kinase C resulting in depletion of NADPH pool. (25)
- Over expression of growth factors. (26)
- Non-enzymatic glycosylation of proteins leading to the formation of advanced glycation end-products. (AGE) (27)

3) Dyslipidemia

Lipoproteins which have been identified as depressing the endothelial function include high levels of LDL, oxidized LDL, lipoprotein (a), small dense LDL, post prandial chylomichron remnant, VLDL, and low levels of HDL-C (28)

4) Smoking

Cigarette smoking has been shown to be associated as a dose-related increase in the intima-media thickness of the vessel and endothelial dysfunction. (29)

5) Obesity

Central obesity with a tendency to visceral fat accumulation is reflected in the waist to hip circumference ratio. The cytokine TNF α , produced by visceral fat adversely affects insulin sensitivity and also has deleterious effects on the endothelium. (30)

6) Aging

Endothelial dysfunction has also been demonstrated in elderly individuals without any other risk factors. (31)

7) Menopause

After menopause, the risk for atherosclerosis increases in females and equals that of males.

This has been attributed to the loss of the “vasculoprotective” effect of estrogens.

8) Hyperhomocystinemia

Homocysteine is a sulfhydryl containing aminoacid derived from the demethylation of dietary methionine. (32) It leads to endothelial dysfunction, accelerated oxidation of LDL – cholesterol, platelet activation and pro-inflammatory response. (33)

9) Mutations in endothelial Nitric Oxide Synthase (eNOS)

Individuals with such a mutation leads to reduce NOformation and accounts for the genetic susptibility of an individual to endothelial dysfunction.

IV ASSESSMENT OF ENDOTHELIAL DYSFUNCTION

During the past decade, a number of methods to assess endothelial dysfunction have been developed.

Most of these techniques make use of the fact that the endothelial cell plays a major role in controlling vascular tone.

A number of mediators take part, of which nitric oxide plays a very important role.

Endothelial function is tested by detecting an increase in blood flow or in diameter of a blood vessel in response to agents that increase the concentration of nitric oxide. (34,35)

The vascular smooth muscle function is assessed by measuring the increase in blood flow or in diameter of the vessel in response to agents that act directly on the vascular smooth muscle cell. eg. Glyceryl trinitrate. (GTN)

The assessment of FMD has to be done in accordance with a standardized methodology as per the guidelines started by the American Collage of Cardiology (ACC) in 2002 give by Corretti et al. (4)

The following recommendations have been made so as to get a correct estimate of the endothelial function.

- Assessment by a single well trained person.
- To withhold vasoactive drugs at least 2 days prior to study.
- To abstain from smoking, alcohol and a high fat diet 6 hrs prior.
- Fasting for 8 hrs prior to study.
- Adequate mental relaxation.
- Study to be performed in a quiet temperature controlled room.
- Consistency of the flow stimulus to be maintained.

This technique can also be used to detect endothelial dysfunction in healthy subject with family history of coronary artery disease (36), apart from in those with the classical predisposing conditions for endothelial dysfunction.

The various methods to assess endothelial dysfunction are

1. Ultrasound of brachial artery

This is the most widely used and noninvasive method to assess endothelial function of the peripheral circulation.

Flow-mediated vasodilatation of the artery is obtained by upper arm occlusion and release.

This results in reactive hyperemia which can be assessed either by measuring the arterial diameter or by measuring the blood flow.

The percentage increase in the brachial artery lumen diameter post shear stress as compared to the resting lumen diameter gives an estimation of the flow mediated dilatation (FMD%) and have the endothelium-dependant function.

The vasodilatation which is evoked after administering direct vasodilator agents such as glyceryl trinitrate (GTN) is the endothelium-independent dilatation and the percentage change in diameter from the resting lumen diameter is called GTN%

2. Carotid intima-media thickness

This is technique that uses high resolution vascular ultrasound to measure the intima-media thickness of the carotid artery:

This is a measure of endothelial dysfunction.

3. Arterial compliance

Large and small vessel arterial vascular compliance can be measured non-invasively by a compound arterial pulse waveform analysis device. It provides a method to quantify alteration in vascular compliance following therapeutic interventions.

4. Venous occlusion plethysmography

Assessment of peripheral vascular resistance using strain-gauge venous impedance plethysmography is another non-invasive test for detection for endothelial dysfunction.

5. Laser Doppler skin perfusion.

This technique is used to determine microcirculatory function. However, it needs further validation.

6. Reactive hyperemia peripheral arterial tonometry

(RH-PAT)

Patients with coronary micro vascular endothelial dysfunction, an early stage of coronary artery disease, show an abnormal blood flow in fingertip which can be measured using a fingertip probe. (38)

7. Coronary angiography (39)

Endothelial function can be tested by examining the angiographic vasodilatory response to intra-coronary infusions of endothelial-dependant and-independent vasodilators.

The infusion of acetylcholine evokes a endothelium-dependant NO-mediated vasodilatory response.

8. Inflammatory markers (40)

Endothelial injury results in release of factors that can be detected in circulation and can be used as markers of endothelial dysfunction.

These are: endothelin-1, VWF, TPA, PAI-1, adhesion molecules (VCAM, ICAM, and P-Selection).

9. In vitro endothelial dysfunction assessment

Endothelial dysfunction can be assessed in small resistance arteries (lumen 100-300 μ) in vitro by surgical biopsies and mounting on wire or perfusion pressure micromyography to test response to agonist stimulating vasodilator substances.

V IMPORTANCE AND LIMITATIONS OF ASSESSMENT OF ENDOTHELIAL FUNCTION

The assessment of endothelial function in both peripheral circulation and the coronary arteries in patients with cardiovascular disease and coronary risk factors provides prognostic information about future cardiovascular events. Several studies have examined this correlation.

Perticone and colleagues (41) observed an impaired forearm blood flow response to Acetylcholine predicted future cardiovascular events in patients with hypertension.

Heiter and colleagues observed a similar relationship in patients with coronary artery disease. (42)

Neunteufl and colleagues (43) observed a relationship between endothelial dysfunction and need for a revascularization procedure.

Another study demonstrated that impaired brachial artery flow-mediated dilatation is an independent predictor of short-term events in high-risk patients undergoing surgery for vascular disease. (44)

Endothelial dysfunction has also been shown to be associated with the development of transplant vasculopathy. (45)

This body of clinical evidence fits well with the current paradigms depicting the endothelium as an organ that integrates signals between the milieu of the vascular wall and the vessel lumen.

In this context, endothelial function should represent an excellent “barometer” of underlying vascular health as it represents an orchestrated response to many known and unknown processes that contribute to the development, progression and clinical expression of atherosclerosis.

Although the present body of knowledge supports the notion that endothelial function has prognostic value of cardiovascular risk, several important issues remain unaddressed. (46)

Most of the published data involves selected patient populations with established risk factors for the disease. These, the extent to which these data can be applied to the general population remains to be determined.

Moreover, clinical studies have largely been retrospective and have applied a variety of methods to assess endothelial function.

These issues are critical for any future plans to use endothelial function as a surrogate marker for cardiovascular risk to effectively target individuals for intensive primary prevention and assess potential new therapies.

VI TREATMENT FOR ENDOTHELIAL DYSFUNCTION

1) L-arginine

L-arginine is the physiological precursor of Nitric oxide, and improves endothelium dependant vasodilatation. (47)

It improves vascular health through generation of Nitric oxide (NO), reduced platelet aggregation, and reduction in homocysteine levels.

(Homocysteine increase the NO Synthase inhibition by asymmetric dimethylarginine ADMA) (48)

Increase in NO generation is evident by the increase in c-GMP and L-citrulline levels)



In one study, one single dose of L-arginine improved flow-mediated vasodilatation of the brachial artery in 35 patients with hypertension. (49)

2) N-acetylcysteine

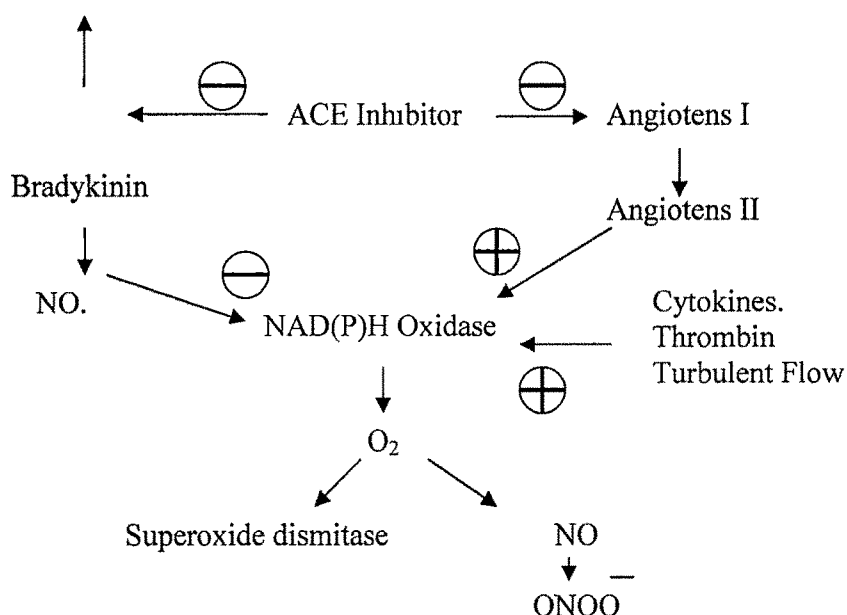
This is a precursor of L-cysteine and potentiates the activity of nitric oxide.

3) Renin – Angiotensin System blocked

The Heart Outcomes Prevention Evaluation (HOPE) study (50) as well as the study to evaluate carotid ultrasound changes with rampril and vitamin E (SECURE) study (51) demonstrated that treatment with an ACE inhibitor had beneficial effects on progression of atherosclerosis.

In these studies, long term ACE inhibition significantly reduced the rate of death from myocardial infarction, stroke and reduced carotid intima-to-media ratio in patients at high risk for cardiovascular events

Inactive Fragments



The Ace inhibitor Lisinopril and the Angiotensin receptor blocker (ARB) Candesartan were shown to acutely improve endothelial dysfunction in human coronary arteries. (52)

4) Statins.

Statins have a number of beneficial effects, some of which are independent of their cholesterol lowering effect, and this would increase the number of disorders in which they would help. (53)

The important effects of statins are

- Improves endothelial function through increased NO production via up regulation of endothelial (eNO) synthase.
- Anti-inflammatory effects by decreasing acute phase reactants, CRP, inflammatory cytokines and cell adhesion molecules.
- Anti-oxidant effects due to scavenging of Superoxide and inhibition of isoprenoids (Superoxide generators) (54)
- Anti-thrombotic effects.
- Stabilization of atheromatous plaque
- Anti proliferative effects due to inhibition of smooth muscle cell proliferation.

! These effects are responsible for the early reduction of cardiovascular events reported even before changes in lipid level occurs. (55)

5) Low fat Mediterranean diet

This consists of salads, citrus fruits and nuts and has shown to lower the risk of cardiovascular events and improve endothelial function. (56)

6) Weight loss

Obesity causes endothelial dysfunction mediated by cytokines TNF- α and interleukin-6.

Weight loss thus improves endothelial function. (57)

7) Exercise

Regular aerobic physical activity improves endothelial function by reduction in blood pressure and improving glucose tolerance.

The JNC – 7 report recommends these life-style changes for all patients with hypertension.

Modification	Systolic BP reduction
-Weight reduction (BMI – 18.5 – 24.9 kg/m ²)	5-20 mmHg
-DASH eating plan (Dietary approaches to stop hypertension)	8-10 mmHg
-Dietary sodium reduction (< 2.4gm sodium/day)	2-8 mmHg
-Physical activity	4-9 mmHg
-Moderation of alcohol consumption	2-4 mmHg

8) Aspirin

Aspirin improves endothelial function by inhibition of the enzyme cyclooxygenase and this leads to vasodilatation and prevention of thrombosis. (58)

9) Antioxidants

Vitamin C, Vitamin –E, and folate has been shown to improve endothelial dysfunction in patients with hypertension. (59)

They act by reducing oxidative stress and preventing the degradation of Nitric Oxide.

Folate, by reducing the level homocysteine helps improve vascular health. Flavonoids found in red wines, grape juice and tea also have anti-oxidant properties and may improve endothelial dysfunction.

10) Estrogens.

Estrogens have been shown to improve endothelial functions in post-menopausal females with hypercholesterolemia

11) Selective Estrogen Receptor modulators

(SERM)

Raloxifene is a SERM that has shown to improve the endothelial function without increase in incidence of Ca breast seen with non-selective estrogens. (62)

12) Newer Modalities

Gene therapy is a novel approach to improve Nitric Oxide production.

An adenovirus mediated endovascular gene transfer of Nitric Oxide synthase (NOS) isoform leads to high level of NOS activity. (63)