Causation, Prevention and Reversal of Vascular Endothelial Dysfunction

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Abstract
Insulin Resistance along with endothelial dysfunction give rise to a constellation of syndromes designated as IRS/MBS metabolic syndrome. Endothelial dysfunction starts early in life much before the development of structural atherosclerosis. Recent insights into vascular biology enable us to understand the molecular mechanisms underlying endothelial dysfunction, and the scope and need for prevention of "pre-clinical" coronary atherosclerosis through lifestyle modification; diet, exercise and stress management.

Diminished production of nitric oxide (NO) and/or increased inactivation of NO through oxidative stress (reactive oxygen species ROS and reactive nitrogen species (RNS) are the basis of endothelial dysfunction hence increasing the bioavailability of NO and decreasing its inactivation is the aim of prevention and reversal of endothelial dysfunction. Insulin regulates constitutive NOS gene expression in endothelial cells in vivo; vasodilation is an important component of Insulin-stimulated whole body glucose uptake.

Successful strategies are: PPAR alpha and gamma agonists which increase NO production in endothelium; antioxidants such as vit. E and C; supplementation with L-arginine, tetrahydrobiopterin-BH4 or sepiapterin (precursor of BH4), SOD mimic tempol, statins which apart from lowering cholesterol improve NO production, selective β1 adrenoreceptor antagonists such as nebivolol; suppression of angiotensin-mediated endothelin production by ACE inhibitors and ATR blockers; CB1 receptor blockers, PKCb inhibitors, nitric oxide donors (glyceryl trinitrate and isosorbide dinitrate), dietary supplements of EPA/DHA and regular physical exercise and control of mental stress.

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INTRODUCTION
Atherosclerotic coronary artery disease (CAD) has assumed epidemic proportions in India, affecting all socio-economic groups. Ethnic Indian populations emigrant to various parts of the world are highly prone to CAD, which occurs in them ten years earlier than the rest of the population, and is more extensive and severe compared to the other ethnic groups of the population. Insulin Resistance, along with endothelial dysfunction give rise to a constellation of syndromes: described as Insulin Resistance Syndrome (IRS), Cardiovascular Dysmetabolic Syndrome (CDS), or simply as Metabolic Syndrome (MBS): visceral obesity (abdominal girth > 90 cm males, > 85 cm females); hypertension, Type 2 diabetes mellitus (T2DM) and atherosclerotic CAD (Fig. 1 and Fig. 2).1

Insulin regulates constitutive NOS gene expression in endothelial cells in vivo.2

Endothelial dysfunction starts early in life, much before the development of structural coronary atherosclerosis. The multiple risk factors that have been identified for coronary atherosclerosis, can be modified by simple measures such as diet, exercise, and stress control. Patients with risk factors for CAD, normal coronary angiograms and no measurable disease by intravascular ultrasound (IVUS), exhibit selective endothelial dysfunction at both the epicardial and microvascular levels.3 These findings emphasize the scope and need for lifestyle management and prevention of "pre-clinical" coronary atherosclerosis.

With N-13 ammonia PET perfusion studies early detection of abnormal coronary flow reserve (normal: five times the resting flow) due to endothelial dysfunction is now possible in asymptomatic men and women at high risk for CAD.4 Coronary artery calcium score (CCS) ascertained by high resolution CT provides an indicator of atherosclerotic burden. CCS > 100 provides an indicator for aggressive lifestyle modification.5

NORMAL ENDOTHELIAL FUNCTION
Blood vessels are not just passive conduits like water
The vascular endothelium is a dynamic structure that normally maintains vasodilation (via endothelium derived relaxation factors nitric oxide (NO), prostacyclin and endothelium derived hyperpolarization factor (EDHF), and maintain coronary blood flow during increased demand. But it is also equipped to cause vasoconstriction instantly if the emergency situation demands it (via locally produced endothelin, PGH2 and thromboxane). A healthy endothelium inhibits platelet and leucocyte adhesion to the vascular surface and maintains a balance of pro-fibrinolytic and prothrombotic activity.

eNOS can constitutively produce both NO and superoxide. Tetrahydrobiopterin (BH4) is an essential co-factor for NO synthesis. BH4 deficiency causes deficient NO production. BH4 stabilises NOS dimers which prevents uncoupling of NOS and subsequent superoxide formation. Increased superoxide production has been demonstrated in coronary arteries.

Superoxide dismutase (SOD) normally bound to the outer surface of endothelial cells protects NO from inactivation by superoxide. This protective mechanism is overwhelmed in oxidative stress. The SOD mimic tempol restores vasodilatation.

Insulin increases NO production via de novo synthetic pathway for BH4 synthesis. Hyperglycaemia-mediated EPC dysfunction is associated with reduced intracellular BH4 concentration, which is reversed by exogenous BH4 treatment.

Nitric oxide inhibits many of the processes that follow endothelial injury such as NFκB activation, monocyte chemotaxis, foam cell formation, leucocyte and platelet adhesion, intimal hyperplasia, vascular smooth muscle cell (SMC) migration and proliferation, expression of adhesion molecules VCAM-1, ICAM-1 and e-selectin, etc.

In patients with coronary atherosclerosis, endothelial vasodilatory dysfunction is not confined only to the epicardial conductance vessels but also extends to the coronary resistance vessels and microcirculation. Excessive inactivation of NO due to increased oxidative stress occurs in smokers, diabetics with increase in advanced glycosylation end products (AGEs) and in hypercholesterolemia. At any level of total and LDL cholesterol the main culprit is the small dense LDL particle which is easily prone to lipid peroxidation and thereby adhere to the proteoglycan of the endothelium. Paraxonase, an antioxidant enzyme located on HDL prevents lipid peroxidation of LDL (hence the protective effect of HDL against the initiation of atherosclerosis).

HDL activates eNOS leading to increased production of NO. A variety of stimuli regulate eNOS activity involving Akt kinase and/or mitogen-activated protein kinase.

Diminished production of NO and/or increased inactivation of NO are the basis of endothelial dysfunction, hence increasing the bioavailability of NO and decreasing its inactivation caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS) is the aim of therapy to reverse endothelial dysfunction.

**DEMONSTRATION OF ENDOTHELIAL DYSFUNCTION**

Endothelial dysfunction may occur in the absence of angiographic or IVUS-detected atherosclerosis in patients with risk factors for atherosclerosis. Animal studies have demonstrated that known risk factors for CAD (hyperlipidemia, hypertension, diabetes) result in impaired endothelium-dependent vascular reactivity before the development of structural atherosclerosis both at the epicardial and microvascular levels. These findings open the scope for prevention of “preclinical” coronary atherosclerosis.

Coronary spasm mediated by sympathetic α adrenoreceptors can occur in angiographically normal coronary arteries, or it may be superimposed on obstructive coronary vessels. Patients with Prinzmetal’s angina, cocaine overdose, and collagen vascular disease like scleroderma are known to have perfusion defects despite normal coronary angiograms. Provocative testing with ergonovine during cardiac catheterization may demonstrate latent coronary artery spasm (as shown by Maseri in 1990).

Syndrome X is defined as stress induced anginal pain with a positive stress test for myocardial ischaemia, with normal coronary angiogram and normal left ventricular
function. This represents “small vessel disease” with reduced coronary flow reserve in the coronary microcirculation as shown by N-13 ammonia PET.

FMD (Flow mediated vasodilatation), an indicator of nitric oxide availability is measured by strain gauge plethysmography in forearm resistance vessels or non-invasively using high frequency ultrasound and hyperemia induced by blood pressure cuff arterial occlusion, a technique described by Correti et al 1995.

Peripheral vascular endothelial function testing by FMD has been shown to correlate well with coronary artery endothelial function.10 Beneficial effects of L-arginine infusion on myocardial endothelial dysfunction during exercise in patients with angina pectoris and normal coronary angiograms have been demonstrated on myocardial perfusion imaging.11 Significant improvement in arm FMD is seen during laughter (15%) and reduction (47%) during mental stress.12

As inflammation accompanies endothelial dysfunction, markers of inflammation like hsCRP, TNFα, IL-6, fibrinogen, sE-selectin, sICAM-1 and sVCAM-1 serve as measures of endothelial dysfunction.

Endothelin, PAI-1, Prostacyclin, TPA, cellular fibronectin and type IV collagen fragments are other markers of endothelial dysfunction.

Microalbuminuria (30-300 mg/day) due to transmembrane leakiness is a marker of generalized endothelial dysfunction. More importantly it represents a stage at which prevention of future cardiovascular and renal deterioration is possible.

**EPC Dysfunction**

Endothelial progenitor cells (EPCs) are critical for endothelial maintenance and repair. Bone marrow derived EPCs home to sites of ischaemia, incorporate into newly formed capillaries and augment neovascularization (important for wound healing and tissue repair) under the stimulation provided by erythropoitin (EPO).

In healthy person the number of circulating EPCs serve as a surrogate marker for vascular function and cumulative cardiovascular risk. EPC dysfunction contributes to the pathogenesis of ischaemic vascular disease. EPCs in circulation are depleted in patients with active rheumatoid arthritis (RA). Endothelial inflammation, characterized by permanent over-expression of cellular adhesion molecules and pro-inflammatory cytokine, CRP, matrix metalloproteinase 9 and IL – 18 are all elevated in RA and are involved in the development of atherosclerosis.13

In patients with cardiovascular risk factors the number and function of EPCs is impaired. EPCs probably secrete angiogenic factors to activate resident endothelial cells. The number of circulating EPCs and circulating angiogenic cells (CACS) are inversely related to the several risk factors for atherosclerosis.

Hyperglycaemia affects EPC proliferation and differentiation. The number of EPCs obtained from T1DM patients in culture was 44% lower compared to age and sex matched healthy controls.14 EPC dysfunction is a novel concept in the pathogenesis of vascular complications of T1DM. Hyperglycemia causes uncoupling of endothelial NOS, impairing EPC mobilization and function.9

Statins are known to increase EPC number, while high TNFα causes decrease in circulating EPCs.

The Emory University, USA is currently assessing the role of Mediterranean diet and omega 3 fatty acids on oxidative stress and endothelial progenitor cells.

Caveolin, the structural protein of calveolae plays a central role in EPC mobilization and homing in SDF-1 driven post-ischemic vasculogenesis.15 It also regulates eNOS turnover and consequently NO release as a result of shear stress.

**ANTI OXIDIZED LDL ANTIBODIES**

Endothelial inflammation is initiated by oxidize LDL, which acts as an immunogen and stimulates the production of auto antibodies by B cells. Anti OXLDL antibodies are present in healthy individuals as well as in patients with atherosclerosis. Circulating IgG antibodies to OX-LDL are present in cardiovascular risk-free children, and the levels of antibody are significantly higher in children, than adults suggesting that the antibodies may not necessarily be related to atherosclerosis and cardiovascular disease, and indeed may be protective against atherosclerosis by neutralizing and catabolizing the OX-LDL.

It is possible that some infections with phosphorylcholine as a major antigen (e.g., streptococcus pneumoniae) give rise to anti phosphocholine antibodies (IgM) which block the uptake of OX-LDL by macrophages. On the other hand circulating immune complexes (CICs) – OX-LDL and antibodies to OX-LDL may cause damage when deposited in tissues including atheromas.

Only after clearly defining the characteristics of protective anti OX-LDL antibodies and the conditions of their generation will we know whether we can immunize patient with OX-LDL to stimulate protective antibodies especially in patients with accelerated atherosclerosis such as SLE, RA and APS and for the prevention of restenosis.16

**EPA AND DHA**

Phospholipase A2 acts on cell membrane phospholipids to release N6 or N3 fatty acids which are then metabolized by cyclo-oxygenases and lipo-oxygenases to eicosanoids. Arachidonic acid (n6 fatty acid) produces prostanoids such as PGI2, TXA2, PGD2, PGE2, PGF2alpha whereas eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) intake reduces production of prostanoids and produces those that are weaker e.g. TXA3, increases the production of protacyclin leading to vasodilatation, inhibition of platelet aggregation and inflammation.17

Longterm treatment with EPA augments both NO related and non-NO mediated endothelin-dependent forearm
vasodilatation in patients.20

Asymmetric dimethyl arginine (ADMA) is an endogenous competitive inhibitor of eNOS. Plasma accumulation of ADMA is a risk factor for endothelial dysfunction. EPA and DHA reduce plasma levels of ADMA and arachidonate.

A study done by Shimojo et al (2006) assessed the effect of EPA on NOS gene expression and on NO level in endothelin-1 induced hypertrophied cardiomyocytes. mRNA expression of iNOS was significantly increased in ET-1 treated cardiomyocytes and was suppressed by EPA treatment, i.e. iNOS expression was suppressed without modulating total NO level or eNOS gene expression.

The EARLY Study19 evaluated the role of DHA in restoring endothelial function in children with hyperlipidemia. Flow-mediated dilatation increased significantly after DHA supplementation compared to baseline, diet alone, placebo and washout phases of study without affecting biomarkers of oxidative stress, inflammation or ADMA.

Higher concentration of omega 3 fat was associated with improved brachial artery dilatation in young adults with cardiovascular risk factors.20

A study on cultured human endothelial cells showed that exposure to DHA leads to reduced expression of cytokine-induced adhesion molecules.

Nuclear factor Kb (NFkB) is a transcription factor involved in induction of inflammatory genes such as inducible nitric oxide synthase, ICAM-1, VCAM-1 and E-selectin. Fish oil decreases the activation of NFkB.

N3 long chain PUFA modulate sympathetic activity. Supplementation of diet with DHA or fish oil for 3 months prevented young students from developing aggression against others at times of stress.21

EPA protects endothelial cells against anoikis, a phenomenon by which ROS lead to detachment of endothelial cells.

Fish oil supplementation has beneficial effects on plaque stability through higher proportions of EPADHA as they result in thicker fibrous plaques, and reduced abundance of macrophages.22

**ADIPOCYTOKINES**

**Leptin**

Recent evidence points to the role of leptin induced oxidative stress in human endothelial cells in vivo with free-radical induced decreased NO. Elevated plasma leptin and TBARS (marker of lipid peroxidation) were higher and plasma nitric oxide metabolites lower in obese hypertensives compared to obese normotensives.

**Resistin**

Resistin, another important adipocytokine, increases superoxide formation and decreases eNOS expression and thereby causes reduced vasodilation. This has been shown in porcine coronary arteries which can be effectively reversed by seleno-L-methionine, an antioxidant. Plasma levels of resistin are increased in obese individuals and may contribute to endothelial dysfunction. The effects of resistin are opposite to adiponectin, another adipocytokine.

**Adiponectin**

Adiponectin, an insulin sensitizing adipocytokine stimulates the production of nitric oxide in endothelial cells using the PI3 kinase dependent pathway. It induces phosphorylation of eNOS at ser 1177 and complex formation of eNOS with heat shock protein HSP90 through activation of AMP-activated protein kinase (AMPK). Adiponectin receptors AdipR1 and Adip R2, expressed on endothelial cells interact with APPL 1, an intracellular protein to mediate the adiponectin-evoked endothelial NO production and vasodilatation. Whether APPL-1 is also involved in other actions of adiponectin such as protection from apoptosis, modulation of cytokine production and alleviation of oxidative stress warrants further studies.23

Hypoadiponectinemia is closely linked to the impairment of endothelial function in HT and Type 2 DM as shown by increase in CRP, PAI-1 and tPA (which impair fibrinolysis). On the other hand, transgenic or adenovirus-induced over expression of globular or full length adiponectin results in marked alleviation of atherosclerosis in ApoE deficient mice. The main benefit of PPAR gamma agonist (such as rosiglitazone) is through increased adiponectin production. Adiponectin activates AMPK in both the liver and skeletal muscle. EPA/DHA induce adiponectin in mice fed a high fat diet.

BH4 oral supplementation (10 mg/kg/day) leads to significant increase in plasma adiponectin and expression of mRNA of adiponectin in adipocytes.24

**ENDOCANNABINOIDS (EC) SYSTEM ACTIVATION**

The endocannabinoid (EC) system, a physiological system normally silent, gets activated under painful and stressful circumstances. It produces on demand fatty acid amides such as arachydonyl ethanolamide (Anandamide) and arachydonyl glycerol (2 AG) which through a central mechanism help to reduced pain and anxiety, produce slowing down, relaxation and sedation, eliminate unpleasant memories, and increase appetite and food intake through a central mechanism, and promote lipogenesis through a peripheral mechanism. Cannabinoid receptors CB1 are expressed in the brain, liver, GI tract, muscle and adipose tissue and vascular endothelium and sympathetic nerve terminals Endocannabinoids induce vasodilatation via CB1 receptor in the arterial smooth muscle in the brain, and other vascular beds through increased NO synthesis (Gelfand E and Cannon C 2006).25 CB2 receptors are expressed on the cells of the immune system whose activation stimulates production of pro-inflammatory cytokines e.g. TNFα, IL-6 etc.

EC system has a role in regulating cardiovascular and metabolic risk factors. Overactivity of EC system is associated with excessive food intake, obesity and insulin resistance. Circulating levels of anandamide and 2AG are
increased (35% and 52% respectively) in obese compared to lean women.

Remonabant, a CB1 receptor antagonist stimulates adiponectin production in adipocytes and helps to reduce weight (as indicated by a fall in leptin levels) in obese patients with T2DM and dyslipidemia. The beneficial effects on cardiometabolic risk factors have been shown in clinical trails.25

**PPAR Agonists**

Peroxisome proliferator activator receptors (PPAR) are nuclear transcription factors that modulate expression of genes that regulate lipid and glucose metabolism and energy storage and expenditure in an organ specific manner. They are of three main types: alpha, gamma and delta. PPAR gamma is predominantly expressed in adipose tissue and regulates formation and function of fat cells.

PPAR alpha agonists such as fenofibrate, apart from lowering serum triglycerides and LDL cholesterol and raising HDL cholesterol, improve vasodilator function by upregulating eNOS expression mainly through mechanisms stabilizing eNOS mRNA. This is a new observation to explain one of the mechanisms of PPAR-alpha mediated cardiovascular protection.26 Clinical studies measuring forearm blood flow have demonstrated that endothelium-dependent vasodilatation is improved by PPAR alpha agonists.

PPAR gamma agonists such as rosiglitazone are shown to improve vascular and endothelial function by increasing NO release from vascular endothelial cells without altering eNOS mRNA levels probably through a transcriptional mechanism unrelated to eNOS expression. Together, PPAR alpha and PPAR gamma may increase NO production in endothelium by different mechanisms.

PPAR gamma agonists also reduce ROS generation and improve flow-mediated vasodilatation in brachial artery.

Synthetic DHA derivatives show PPAR gamma transactivation higher than or comparable to that of pioglitazone, a PPAR agonist.27

The expression of eNOS and endothelin-1 is coordinately regulated in health and disease. PPAR alpha agonists have the potential to inhibit endothelin expression by inhibiting the activator protein 1 (AP1) signalling pathway.

PPAR gamma agonists also suppress endothelin.

PPAR delta agonists are promising new molecules which will prevent weight gain (unlike PPAR gamma) by expressing UCP genes in skeletal muscle and adipose tissue. Bezafibrate is a pan-PPAR activator.

**C Peptide Deficiency and Endothelial Dysfunction**

A biological role for C peptide was suggested by the clinical observation that T1DM patients with some measurable C peptide have less long term complications than T1DM totally deficient in C peptide. Islet cell / pancreatic transplants restored both insulin and C peptide resulting in amelioration of diabetic nephropathy and neuropathy. Short term C peptide infusion as well as oral replacement therapy for 3 months was shown to reduce albuminuria by 40%, and nerve conduction velocity was improved by 80% (changes in glycemic control could not account for the improvement). It is now shown that C peptide at physiological concentrations (0.9 nmol/l) releases nitric oxide (NO) in endothelial cells in a time and concentration dependent manner and increases intracellular Ca2+. The effects are abolished by pertussis toxin, suggesting Gi/Go linked protein interaction in C peptide signaling. It should be emphasized that the benefits are seen in C peptide deficient status only.28

Excess of C peptide, like excess of insulin and proinsulin, can have undesirable consequences through activation of several protein kinase isoforms (MAP kinase and NFKB activation).

**Hyperhomocysteinemia**

Homocysteine > 15 mg % reduces NO bioavailability by oxidative stress. Homocysteine may cause ADMA accumulation by inhibition of DDAH.

In the Indian population, hyperhomocysteinemia is an indicator of widely prevalent deficiency of Vit B12 and folic acid which must be promptly corrected as emphasised by Yajnik et al.2006. Hyperhomocysteinemia as a primary risk factor should be considered in individuals with atherosclerosis at young age, or out of proportion to established risk factors.

**EDHF**

Endothelium derived hyperpolarisation factors (EDHF) are compounds responsible for the NO and prostacyclin-independent but endothelium dependent vasodilatation of some vascular beds. A Cytochrome P450(CYP)-dependent, EDHF response has been described in mammary artery, forearm vasculature and thigh skeletal muscle circulation. In human mesenteric and renal arteries, EDHF mediated relaxation is independent of CYP activity. Epoxyeicosatrienoic acids (EETs) are epoxides of arachidonic acid generated by CYP epoxygenases. They also exert membrane potential-independent effects and modulate several signaling cascades that affect endothelial cell proliferation and angiogenesis. CYP expression and EET generation are increased in hypertension, hypercholesterolemia and salt loading. In addition to EETs, CYP 2C epoxygenase generates superoxide radicals, inhibition of which in CAD improves acetylcholine-induced vasodilatation in forearm vasculature (Fleming I, Busse R 2006).29 EDHF system provides a new approach to treat CAD, stroke and Raynaud’s disease.

Women and CAD

Many women with chest pain with normal coronary angiogram have abnormal myocardial flow reserve due to small vessel disease with endothelial dysfunction. Chest discomfort that is atypical for angina pectoris is
more common in women than in men, mainly due to microvascular and vasospastic coronary artery disease. Women with epicardial coronary artery lesions more often report chest discomfort at rest, during sleep or during mental stress than in men. It is very easy to dismiss it as non-cardiac pain and deny them further confirmatory testing. This happens all the time in all parts of the world.

Anti-endothelial cell antibodies (AECAs) and antiangioplipid antibodies (ACLAs) in young patients with peripheral atherosclerotic disease, have been demonstrated by several groups.

ACLA and/or AECAs were present in 24% of young patients with peripheral vascular atherosclerosis and 60% of them were females, and there was a lower prevalence of dyslipidemia in patients with autoantibodies in comparison with the rest of the patient group. It remains to be determined if the antibodies are mere indicators (secondary to vascular damage) or causative of damage. In young women with no smoking history and hyperlipidemia, a causative role can be postulated for these antibodies.

**Ischaemia Induced By Mental Stress**

John Hunter, the famous British surgeon who had angina pectoris, was well aware of the effect of anger when he said, “my life is in the hands of any rascal who chooses to provoke me”. Effects of anger on left ventricular ejection fraction (LVEF) have been well documented. Sudden cardiac death has been triggered by an earth quake.

More often, mental stress induces silent ischemia as first demonstrated by Deanfield in 1984 and several other studies. Mental stress-induced ischemia has been reproduced in the laboratory and has been compared with ambulatory ischemia during daily life along with haemodynamic features.

It is important to note that mental stress can induce silent ischemia more frequently than physical exercise in the same patient. Laboratory protocols involving mental stress (arithmetic problems, speaking assignments) have been used along with continuous monitoring of LVEF by ambulatory vest. The prognostic value of mental stress testing in CAD has been established. Mental stress can induce adverse cardiac events in stable angina patients.

FMD studies have shown significant reduction (47%) in forearm blood flow during mental stress, and significant improvement (15%) during laughter.13

A study of men in depressive mood (4-12 score on the 13 point Welsh depression subscale) has shown 46% higher hsCRP, 16% higher IL-6 and 10% higher ICAM-1 expression compared to controls.30

Arachidonic acid (n6 PUFA) is a precursor of proinflammatory cytokines and eicosanoids whereas EPA/DHA (n3 PUFA) inhibit them. Therefore it was proposed that by altering membrane fluidity and reducing proinflammatory cytokines and eicosanoids, omega 3 fat could have a beneficial effect on neurotransmission and depression. This has been supported by various studies.31

Myocardial stunning due to sudden emotional stress has been documented.32 Neurohumoral assessment of such patients has shown high levels of plasma catecholamines, neuropeptide Y, BNP and serotonin, suggesting sympathetically mediated microcirculatory dysfunction. Women appear to be more vulnerable to sympathetically mediated myocardial stunning which has been described after subarachnoid haemorrhage and stroke.

Silent ischemia and silent myocardial infarction

Clinicians often face the enigma of ‘silent myocardial ischemia, first recognized by 24 hour Holter ECG monitoring – definite ECG changes while the patient does not experience any pain in the chest were shown by Stern and Tzivini in 1974. It is important to note that the prognosis of myocardial ischemia is not affected by the presence or absence of concomitant angina. In fact sudden unexpected death is the first and only “symptom” in more than 25 percent patients of CAD. Between 20-60% of non-fatal acute myocardial infarcts are not recognized by the patient; of these about half are truly silent, with the patient unable to recall any symptoms whatsoever; silent infarct occurs more commonly in patients without antecedent angina pectoris, and in patients with diabetes and hypertension.

**How To Improve Endothelial Function?**

Dean Ornish set up a study in 1977 to explore the effect of diet and lifestyle modification on the reversal of coronary atherosclerosis, using the techniques of quantitative Coronary Arteriography and quantitative myocardial blood flow using N-13 ammonia PET imaging. The findings were published in JAMA (1983), Circulation (1989), Lancet (1990), JAMA (1995) and JAMA (1998). It became obvious that modification of cardiovascular risk factors that contribute to endothelial dysfunction improve patient outcome disproportionately to the regression in the anatomic atherosclerotic lesions. Patients with “mild” coronary artery lesions can have severe endothelial dysfunction. Long term follow-up showed more cardiac events in such patients. Myocardial ischemia can occur during exercise in patients without significant epicardial vessel stenosis, due to endothelial dysfunction. Recent insights into vascular biology help us to understand how the benefit occurs in relation to endothelial dysfunction.

A single high sugar intake may induce heart rate acceleration and blood pressure elevation as a result of sympathetic activation secondary to insulin response and alteration in endothelial function due to activation of oxidative stress.34 Hyperglycemia activates protein kinase C (PKC-b) which depresses eNOS expression and increases endothelin-1 activity. Treatment with vitamin E inhibits PKC, by increasing enzymatic degradation of diacylglycerol (DAG), the source of PKC, or by activating protein phosphatase 2 A which dephosphorylates PKC. This action may be independent of the anti-oxidant effect of vitamin E.
Endothelial dysfunction caused by vascular PKCb activation due to hyperglycaemia is critical for diabetic microvascular complications. This can be prevented by selective PKCb inhibitors such as Ruboxistaurine.35

A single high fat meal transiently reduced endothelial function for up to 4 hours in healthy, normocholesterolemic subjects, probably through accumulation of triglyceride rich proteins. This decrease was blocked by pretreatment with antioxidants vitamin C and E, suggesting an oxidative mechanism which increases superoxide production and deactivates nitric oxide.36

Lipid lowering agents and antioxidants provide more nitric oxide and reduce superoxide thereby enhancing endothelial function, reduce thrombotic potential, reduce inflammatory response thereby preventing rupture of the vulnerable plaque. Clearly mechanisms other than regression of the atherosclerotic stenosis are operating.

Statins, apart from lowering cholesterol also improve nitric oxide production. Patients with low hsCRP do not get significant benefit with statin therapy compared to those with high hsCRP.

Advanced glycosylation end products (AGEs) through their receptors on the endothelium contribute to endothelial dysfunction. Impairment of endothelium-dependent vasodilation in Type 2 DM occurs independent of epicardial coronary atherosclerosis, and cause major cardiac events. Strict control of diabetes reduced the AGES.

Nitrates (glyceryl trinitrate, isosorbide dinitrate) act as nitric oxide donors and thereby improve endothelial function apart from decreasing the preload and afterload of the ventricles. Long term use of nitrates can produce drug tolerance which can be overcome by a combination of nitrate and hydralazine.

Newer selective β1 adreno-receptor antagonists such as nebivolol inhibit endothelin-1 liberation and increase the availability of nitric oxide.

ACE-inhibitors and Angiotensin receptor blockers prevent angiotensin-induced endothelium and superoxide production. ACE inhibitors improve endothelial function in subcutaneous, epicardial, brachial and renal circulation, whereas they are ineffective in potentiating the blunted response to acetylcholine in the forearm of patients with essential hypertension. They can also selectively improve endothelium-dependent vasodilatation to bradykinin, probably related to hyperpolarisation. Treatment with an AT(1)-receptor antagonist can improve basal NO release and decrease the vasoconstrictor effect of endogenous endothelin-1. Calcium channel blockers can reverse impaired endothelium dependent vasodilatation in subcutaneous, epicardial, renal and forearm circulation.

Nifedipine and lacidipine can improve endothelial dysfunction in the forearm circulation by restoring NO availability probably through an antioxidant mechanism.

Tetrahydrobiopterin (BH4) oral tablets are available but are prohibitively expensive hence no clinical trials have been undertaken. BH4 supplementation prevent endothelial dysfunction and restores adiponectin levels, as described in an earlier section. Administration of sepiapterin (precursor of BH4) restores coronary flow reserve.37 In healthy adults, oral-glucose induced hyperglycemia and endothelial dysfunction is reversed acutely with BH4 infusion; the same benefit is seen in type 2 DM.

Normal diet contains 8 gm L-arginine. An additional 8 g/day nutritional supplement on a long-term basis improves endothelial function.38

Erythropoietin (EPO) promotes endothelial progenitor cell proliferation and adhesive properties in a PI3 kinase dependent manner. Recombinant human EPO protects the myocardium from ischemia–reperfusion injury and promotes beneficial remodeling, a novel protective effect in the infarcted heart.

The vascular action of insulin to stimulate endothelial production of NO leading to vasodilation and increased blood flow is an important component of insulin-stimulated whole body glucose utilisation.

Exercise training improves coronary flow reserve as a result of improved vasodilation. By increasing shear stress, it induces increase in NO, largely dependent of activation of tyrokinase CSR-C expression. Regular physical exercise improves insulin resistance in muscles and improves glucose uptake and glycogen synthesis.

The fatty acid composition of skeletal muscle membrane phospholipid is altered by N-3 PUFA, increasing its fluidity, thereby permitting prolonged residence of GLUT-4 in the plasma membrane.

A daily dietary supplement of essential fatty acids EPA and DHA is recommended.

Role of DHA in stress management by modulation of sympathetic activity in the CNS has already been discussed in an earlier section.

Yoga and life style have shown reversal of atherosclerosis. A prospective study done by the Caring Heart Project of International Board Yoga (2003) has shown the beneficial effects of Yoga Lifestyle on reversibility of ischaemic Heart disease.39

Need for aggressive revascularization

In Harrison’s Principles of Internal Medicine 17th ed. 2005, p. 1304, Eugene Braunwald, eminent cardiologist has stated: “Mechanical revascularization by CABG (Coronary Artery bypass graft) surgery or PTCA (percutaneous trans thoracic coronary angioplasty) with or without drug-eluted stents, is probably being employed too often in USA. The mere presence of angina pectoris and/or the demonstration of severe coronary artery narrowing at angiography should not reflexly evoke a decision to do aggressive revascularization. Instead, this approach should the limited to those patients whose angina has not responded adequately to medical treatment or in whom revascularization has been shown to improve the natural history of the disease e.g. acute
coronary syndrome, or multivessel CAD with left ventricular dysfunction (LVEF < 40%), and hibernating myocardium.

Unfortunately commercial pressures world-wide have distorted the scientific practice of medicine. Dean Ornish has said in his book “Reversing Heart Disease” “The insurance company will pay at least $ 30,000 for a CABG, at least $ 7500 for a balloon angioplasty, but only $ 150 if a doctor spends the same amount of time and effort educating a heart patient about nutrition, exercise, stress-coping and, life style management. If some one spends the same amount of time and effort teaching a well person how to remain healthy, the insurance company will pay nothing at all. It is nor surprising that doctors spend time doing what is reimbursed”.

**SUMMARY AND CONCLUSIONS**

By the year 2025 half the world’s population of type 2 DM will be in India. A polypill strategy (aspirin 75mg, metformin 1gm, ACE inhibitor 10mg and statin 40mg) per day can slash diabetic risks and reduce subsequent events by up to 97 percent over 5 years.

In the larger long –term interest of Indian Society in the next 25 years our emphasis should be on prevention through lifestyle modification beginning from childhood. A vegetarian diet with fat not more than 15%, adequate source of long chain n-3 essential fatty acids EPA and DHA, low sodium, high potassium and high fibre (green vegetables and fruits 400 g/day which also give antioxidants – carotenoids, flavonoids, lycopene, Vit. C, Vit. E) regular physical exercise, avoidance of tobacco and alcohol, and controlling mental stress through Yoga and meditation. Chronic life stressors such as perceived isolation, lack of social support, anxiety, depression, hostility and anger contribute to increased risk of CAD in part by impairing endothelial function. On the other hand positive emotions such as mirthful laughter have the opposite effect. How to keep mental equanimity and how to give and receive love and affection more freely is the key to a happy, healthy life.

**REFERENCES**


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**Announcement**


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