Role of Diet, Exercise and Drugs in Modulation of Endothelial Cell Dysfunction

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Introduction

Endothelial cell dysfunction is the earliest change in vascular endothelium in many diseases, particularly cardiovascular, cerebrovascular and peripheral vascular disease. A variety of risk factors such as diabetes mellitus (DM), hypertension (HT), dyslipidemia, smoking, obesity, hyperhomocysteinemia, lack of exercise, sedentary and poor life style, unhealthy diet adversely affect endothelial function. Emerging evidence indicates an important role of dietary factors in modulating endothelial function either by decreasing endothelial activation or by improving endothelial dependent vasodilation in patients at high risk of cardiovascular disease as well as healthy persons.1

Histologically endothelium was considered as a static monolayer of cells acting as a semipermeable barrier between blood and body tissues.2 Over the past few decades, experimental and clinical evidence has shown that the endothelium is an active and dynamic largest endocrine organ involved in maintaining homeostasis in both healthy and diseased states3. The main functions of the endothelium are to maintain blood circulation and fluidity, regulate vascular tone and modulate leukocyte and platelet adhesion and leukocyte transmigration.

The endothelial monolayer is also important for smooth muscle cell function, vascular remodeling and the maintenance of vascular tone through both vasoconstriction and vasodilation.2 The endothelium synthesizes several molecules that are crucial for its vasomotor function and these can be released in response to local mechanical stimuli (eg flow and shear stress), metabolic conditions (eg hypoxia). The vasoconstrictors are endothelial thromboxane A2, and endothelin -1.4

The endothelium derived vasodilators include NO, endothelium derived hyperpolarizing factor and prostacyclin. Vascular tone is determined by the balance between vasoconstricting and vasodilating agents.2

Nitric oxide is vasodilator, it inhibits platelet aggregation,5 modulates leukocyte endothelium interaction by altering cell adhesion molecule expression and reducing monocyte adherence6 and inhibits the proliferation of smooth muscle cells.

A non-invasive ultrasound technique has been developed in response to increased flow induced by reactive hyperemia7. Anderson et al8 showed that brachial vasodilator response to reactive hyperemia assessed by an ultrasound technique is closely related to the coronary vasodilator response to acetylcholine.

Role of dietary factors that influence endothelial function are:

1. Intake of n-3 fatty acids and risk of CV disease

Cardiovascular disease (CVD) is very rare in populations with a very high intake of fish, such as Alaskan Native Americans,9 Greenland Eskimos10 and Japanese of fishing villages11 which raises the possibility that fish oil is protective against atherosclerosis.

Prospective cohort studies evaluated the association between fish consumption and the risk of CVD in different populations. The results of different fish eating populations confirmed that fish intake is probably more protective against coronary heart disease (CHD) than against non fatal myocardial infarction (MI). Two interventional studies, the Diet and reinfarction Trial (DART)12 and the GISSI – Prevenzione trial 13 evaluated whether fish consumption or fish oil supplementation reduces coronary mortality in MI patients. The results of both studies support the notion that increased consumption of n-3 fatty acids reduces mortality in high risk patients.14

Effects of n-3 fatty acids on endothelial function

There is growing evidence regarding the effect of fish oil on endothelial function.15 Overall experimental studies showed that n-fatty acids particularly DHA, decrease expression of VCAM-1 on the vascular endothelium and decrease leukocyte rolling and adhesion to the endothelium. In an interesting ex-vivo study,16 hypercholesterolemic patients and control subjects received either 10 gm fish oil capsules or placebo for 3 months. Before and after 3 months of supplementation, a sample of skin and glutal fat was biopsied, small arterial segments were removed and vasodilation was assessed in response to acetylcholine (endothelium dependent) and nitroprusside (endothelium independent). Peripheral small arteries from the hypercholesterolemic patients showed significant improvement in endothelium dependent relaxation after fish oil supplementation compared with those from the control subjects.

Two studies examined the effects of n-3 fatty acid supplementation on endothelium dependent vasomotor function. In one study Fleischhauer et al17 assessed the effects of dietary supplementation of 5g EPA plus DHA on endothelium dependent vasodilator responses of coronary arteries to intracoronary acetylcholine infusion in heart transplant recipients. After 3 weeks of treatment, patients treated with fish oil improved their vasodilator response to normal levels, whereas control patients showed vasoconstrictor response. In another study, Goodfellow et al18 randomly assigned 30 hypercholesterolemic subjects to a treatment group with n-3 fatty acids at a dosage of 4g/dl or to a placebo group. At baseline hypercholesterolemic patients showed impaired endothelium-dependent vasodilation compared with healthy subjects. After 4 months
of treatment, the patients supplemented with n-3 fatty acids had significantly improved endothelium dependent vasodilation compared with control subjects.

2. Fatty acids

Vogel et al. showed that high fat meal containing predominantly saturated and transfatty acids induced acute decrease in flow mediated dilatation (FMD) that correlated with a postprandial elevation of triglyceride rich lipoproteins in the plasma. The chronic consumption of low fat diets and Mediterranean-style diets improve endothelial function compared to a high fat western type diet.

In conclusion, with the exception of n-3 fatty acids, acute or chronic high fat meals induce a negative effect on endothelial function. This function is improved by the simultaneous administration of natural antioxidants such as red wine, fruits and vegetables. Studies with fish oil further support the beneficial effects of these fatty acids on endothelial function and cardiovascular disease prevention.

3. Antioxidant vitamins

There is enough epidemiologic evidence linking intake of antioxidant vitamins particularly vitamin E, vitamin C with reduced risk of coronary artery disease. The Nurses Health Study and the Professionals Follow up Study showed that vitamin E consumption caused reduction in risk of CHD. Kenk et al. reported an inverse association between dietary vitamin E intake and coronary mortality in healthy persons. The Iowa women’s study showed that dietary vitamin E intake as opposed to supplemental vitamin was inversely associated with risk of death from CHD. Losonczy et al. found a reduced risk of all cause mortality and coronary artery disease mortality with increased vitamin E intake (including dietary and supplements). They also found that supplementation with both vitamin E and C further reduced the risk suggesting a synergistic effects of vitamin E and C.

However 4 clinical trials regarding the effects of vitamin E on risk of CVD such as The Cambridge Heart Antioxidant study (CHAOS). The Alpha-Tocopherol Beta-carotene Cancer Prevention study (ATBC). The GISSI – Prevenzione trial and the Heart Outcomes Prevention Evaluation (HOPE) study have shown variable results. The overall results of the clinical trials are not that encouraging given the consistent and promising findings from epidemiologic studies. The antioxidants reduce the susceptibility of LDL oxidation and scaveng free radicals within the body, reducing overall oxidative status of a person. Experimental studies have shown that anti-oxidants improve endothelium dependent vasodilation after incubation with antioxidants. In addition to in vitro evidence, there is enough clinical evidence to support favorable effects of anti-oxidants on endothelial function.

Apart from vitamin E, vitamin C and probucol, flavonoids and other polyphenols containing foods such as red wine, tea, onions, garlic, apple and other fruits also have anti-oxidant properties as their intake is associated with reduced CV risk. Short and long term black/green tea consumption reverses endothelial dysfunction in CAD patients.

4. Folic acid and vitamin B12

Several epidemiologic studies examined the association between folate intake and CVD. The Nurse’s Health Study which included 80082 women who were followed up for 14 years, folate intake caused significant risk reduction. Also Chee and Stamler reported significant inverse association between folate intake and mortality from all causes and CVD in 6426 men from the Multiple Risk Factor Intervention Trial (MRFIT) usual care group. The primary mechanism for the effect of folic acid is a reduction in plasma homocysteine which causes endothelial cell dysfunction. Woo et al. showed that 10 mgm folic acid for 8 weeks significantly improved flow mediated endothelium dependent vasodilation in 17 healthy persons with relative hyperhomocysteinemia. The overall results of different studies suggest that folic acid has a beneficial effect on endothelial function as measured by flow mediated vasodilation in healthy persons or patients with hyperhomocysteinemia.

The beneficial effect of folic acid is mediated by homocysteine lowering effect of folic acid and by other mechanisms such as anti-oxidant properties and direct NO production. Folate and vit. B12 improved insulin resistance and ECD along with lowering of homocysteine.

5. L-arginine

There are several reports of L-arginine having beneficial effect on endothelial function. Taken together, the results of these studies suggest a potential effect of L-arginine on endothelial function in patients with hypercholesterolemia or existing coronary artery disease. In several large epidemiologic studies, a high consumption of nuts (high arginine content) was associated with a significantly lower risk of CHD. In patients with CAD, most studies have shown that L-arginine improves both endothelium dependent vasodilation and abnormal interactions of vascular cells, platelets and monocytes. L-arginine shows beneficial effects in patients with dyslipidemia or cigarette smoking.

6. Soy Protein

Dietary soy protein has several beneficial effects on CV health. It causes reduction of cholesterol and triglycerides concentrations. In addition soy protein has anti-oxidant properties. These effects have been attributed to soyabean compounds eg isoflavones mainly genistein. In vitro studies showed that genistein relaxes rat arteries by a NO-dependent mechanism, thus suggesting that these isoflavones could have direct beneficial effect on endothelial function.

7. Mediterranean Diet, life style factors, and 10 year mortality in elderly European men and women. The Hale Project

Mediterranean diet, moderate alcohol consumption, moderate to high physical activity levels, non-smoking were associated with lower mortality rates from all causes, CHD, CVD, cancer and other causes during the 10 year follow up. One may conclude that diet rich in fats, red and processed, meats, sweets, desserts, French fries and refined grains cause endothelial cell dysfunction. Whereas diet rich in fruits, vegetables, legumes, whole grains, fish, olive oil, L-arginine, folic acid, soya protein and anti-oxidants (vitamins C, E, Beta carotene, leutin, selenium), high fiber diet, red wine, black/green tea, almonds, walnut improve endothelial cell function.

Exercise and Endothelial Function

Aerobic exercise, one of life style modifications, reduces
cardiovascular morbidity and mortality. Exercise training improves endothelial function in animal models of hypertension and in patients with essential hypertension. These findings suggest that endothelial dysfunction in hypertension is reversible. Exercise increases NO production and decreases NO inactivation, leading to an increase in NO bioavailability.40

Regular physical exercise which is known to promote a favorable cardiovascular state, improves endothelial function via several mechanisms. It augments blood flow and laminar shear stress, resulting in increased nitric oxide production and bioavailability. The beneficial effects of exercise on endothelial function can be mediated in a number of ways, including synthesis of molecular mediators, changes in neurohormonal release and oxidant/antioxidant balance. In addition exercise can also elicit systemic molecular pathways connected with angiogenesis and chronic anti-inflammatory action with consequent modification of the endothelial function. However benefit depends on the type and intensity of exercise. While strenuous exercise increases oxidative metabolism and produces a pro-oxidant environment whereas regular moderate exercise promotes an antioxidant state and preserves endothelial function. Thus exercise may have a beneficial effect on the development of cardiovascular disease through preserving endothelial function.40

Regular physical exercise is associated with beneficial changes in blood pressure, lipid metabolism, glucose metabolism, neurohormonal factors, body weight and shear stress. (Martin et al.,41 Wood et al.,42 Arakawa,43 Paffenbargery et al.).44 Although the mechanism of improvement in endothelial function during exercise has not been fully clarified, it is thought that regular aerobic exercise increases nitric oxide (NO) production with up regulation of endothelial NO synthase (eNOS) gene expression and vascular endothelial growth factor (VEGF) induced angiogenesis. It also decreases NO inactivation with augmented antioxidant system, such as superoxide dismutase (SOD) and glutathione peroxidase (GP)’s and attenuation of nicotinamide adenine dinucleotide phosphate (NADH/NADPH) oxidase activity, leading to an increase in NO bioavailability.

Epidemiologic Studies on Exercise

Epidemiologic studies have shown that daily exercise such as walking, jogging, cycling or swimming lowers blood pressure. It is clinically important to select the appropriate intensity, duration, frequency and kind of exercise because intense exercise can be hazardous to human vessels. (Abraham et al 1997,45 Bergholm et al, 1999).46 The moderate intensity exercise fits the index of exercise training that is recommended from the preventive general view point of cardiovascular diseases.

Exercise and Endothelial Function

Physical exercise enhanced endothelium dependent vaso dilatation in forearm circulation in hypertensive patients (Higashi et al.,47 Goto et al48). The authors have reported that long term moderate intensity exercise but not mild or high intensity exercise augmented endothelium dependent vasodilation in healthy subjects (Goto et al, 2003).48 A large number of studies have shown that even in normal control animals (Wang et al,49 Sessa et al50 and Bernstem et al)51 and healthy subjects (Green et al,52 Kingwell et al,53 exercise augments endothelial function.

Increase in Nitric Oxide Production

There is an increase in NO bioavailability (increase in NO production and/or decrease in NO inactivation). Exercise training, probably by an increase in shear stress, exerts its beneficial effects on endothelial function by activation of several signal transduction pathways (Traub and Berk).54 Heat shock proteins (HSP) are present in mast cells, including endothelial cells and play an important role in cellular homeostasis and cell protection from damage in response to stress stimuli (Garcia-Cardina et al).55 Exercise is a physiological stimulus factor of HSP. Several investigators have focused on the interaction of eNOS with HSP90 (Gracia Cardena et al,55 Fleming and Busse,56 Russell et al).57

Vascular Endothelial Growth Factor : Angiogenesis

Exercise training increases capillary density and the capillary-to-fibre ratio in skeletal muscles in humans (Hudicka et al).58 Various angiogenetic factors, such as VEGF and fibroblast growth factor (FGF) play an important role in angiogenesis in animals as well as in humans (Lee and Feldman).59

Decrease in Nitric Oxide Inactivation (Oxidative Stress)

It has been shown in animals and humans that endothelial dysfunction is associated with an increase in reactive oxygen species (ROS) (Dijhorst-Oei et al,60 Romero and Reckelhoff).61 Several findings suggest that decrease in NO interaction contributes to improvement in endothelial function. The action of increased ROS that inactivates NO was removed by increased NO production. Moderate intensity exercise predominantly increases NO production compared with ROS production, leading to augmentation of endothelial function in healthy subjects.

Nicotinamide Adenine Dinucleotide (NADH), Nicotinamide Dinucleotide Phosphate Oxidase (NADPH)

NADH/NADPH oxidase is the most important source of superoxide in the vasculature (Cai and Harrison,62 Sowers).63 It is thought that inactivation of NADH/NADPH oxidase may contribute to the improvement in endothelial cell function (ECF) after exercise. These findings suggest that aerobic exercise may improve ECF through decrease in ROS production with inactivation of NADH/NADPH oxidase.

Vasoconstrictors

Longterm aerobic exercise significantly reduced plasma norepinephrine concentration in patients with hypertension (Higashi et al).44 Regular exercise may play an important role in protection of the endothelium through reduction in norepinephrine, leading to augmented Ach-stimulated NO release in hypertensive patients. Exercise training may augment endothelial function through a decrease in vasoconstrictors.

Prostaglandins and Endothelium Derived Hyperpolarizing Factor

Other endothelium dependent vasodilators, such as prostaglandins and endothelium-derived hyperpolarizing factor (EDHF) may also contribute to exercise induced vasodilation. Griffin et al65 showed that exercise improves endothelium dependent vasodilation in the coronary artery of the swine after
chronic coronary occlusion through an increase in production of NO and EDHF.

One may conclude that beneficial effects of exercise, such as lowered lipoprotein level, increased shear stress, reduced vasoconstrictors and lowered blood pressure, may independently contribute to improvement in endothelial function through increase in NO release and/or inhibition of NO degradation. In healthy persons, shear stress induced increase in eNOS activity predominantly contributes to the augmentation of endothelial function during exercise.

**Drugs and Endothelial Function**

Shindle et al have discussed the drugs to improve endothelial function. Most important molecule NO is potent vasodilator, it also inhibits platelet aggregation, vascular smooth muscle proliferation, leukocyte adherence and LDL exudation all of which are atherogenic. Endothelial nitric oxide synthetase (eNOS) is responsible for majority of endothelium derived production of NO from the substrate L-arginine and the cofactor NADPH and tetrahydrobiopterin. It is well known that conditions which contribute to CVD such as DM, HTN and dyslipidemia work in large part by disruption of endothelial function and increased production of reactive oxygen species (ROS) in endothelial cells. A variety of drugs eg ACE inhibitors (ACE1), angiotensin receptor blockers (ARB), statins, insulin sensitizing agents, beta-blockers, calcium channel blockers, endothelin receptor antagonists, L-arginine and various antioxidants improve endothelial cell function.

**ACE-inhibitors and Angiotensin Receptor Blockers**

Both ACE1 and ARBs have been found to exert positive effects on vascular endothelial function independent of their anti-hypertensive effects. ARBs protect endothelium by reduction of the activity of the NO antagonist endothelin-1.

**Lipid Lowering Agents**

Several large scale studies have shown that statin therapy may improve prognosis in patients at risk for CVD. Statins upregulate by activation of eNOS and thus improve endothelial cell function. Statins also inhibit several cholesterol intermediaries which enhance oxidative stress and vascular smooth muscle sensitivity to calcium. Statins increase NO production, stabilize plaque, inhibit platelet aggregation, improve blood flow. Fenofibates significantly change lipo-protein levels and improve FMD.

**Insulin Sensitizing Agents (Pioglitazone)**

Thiazolidinediones decrease lipid peroxidation, inhibit NADPH oxidase activity, decrease plasma levels of NOS inhibitor asymmetric dimethyl L-arginine and reduce C reactive protein (CRP) levels and Inter Cellular Adhesion Molecule 1 (ICAM1) expression.

**Biguanides (Metformin)**

Metformin activates adenosine monophosphate activated protein kinase (AMPK) which increases the expression of both eNOS and neuronal NO synthase (nNOS).

In human studies, metformin has been shown to enhance brachial artery endothelial function in NIDDM.

**beta Blockers**

Only Nebivolol enhances arterial blood flow by NO mediated mechanisms, suggesting that this particular betablocker has beneficial effect on endothelial function. It relaxes smooth muscle by L-arginine – NO and cyclic GMP pathway.

**Calcium Channel Blockers**

Amongst calcium channel blockers, Nifedipine improves endothelial dependent vasodilatation in patients with CAD and hypercholesterolemia, effect not observed with amlodipine. Long acting nifedipine reverses ECD and causes vasculoprotection by mechanisms independent of Ca channels as endothelial cell membranes do not have Ca channels. It antagonizes effects of endothelin-1 and reduces LDL-C deposition in sub-endothelial layer.

**Endothelins Receptor Antagonists**

Endothelins are a family of vasoconstrictor peptides synthesized by vascular endothelium and smooth muscle cells. ET-1 is released from the endothelium of the corpus cavernosum and is thought to play a role in cavernous artery vasoconstriction. Endothelin Receptor Antagonists (ERA) are drugs that block this effect by interaction with endothelin receptor. Examples include sitaxsentan, a selective endothelin A receptor blocker and bosentan, which blocks both endothelin A and B receptors.

**L-arginine**

L-arginine is the substrate from which eNOS produces NO. Supplementation of L-arginine boosts NO production. L-arginine improves coronary and peripheral vasodilation in hypercholesterolemic patients via endothelium dependent mechanisms.

**Anti-oxidants**

Anti-oxidant vitamins are scavengers of reactive oxygen species (ROS) and thus they prevent conversion of NO to peroxynitrite and increase NO bio availability. Vitamin E or tocopherol inhibits LDL oxidation and leukocyte adhesion in vitro. Vitamin E in combination with simvastatin improves flow mediated brachial artery dilation more than simvastatin alone.

**Probucol**

It improves brachial artery flow mediated dilatation.

**L-carnitine**

L-carnitine confers benefits in patients with CVD, exerting improvement in endothelial function manifested by increased blood flow.

One may conclude that potential for endothelial active drugs to modulate endothelial dysfunction is important. ACEI and ARB appear to hold great promise in this regard. Whereas statins and insulin sensitizers may play a potentially useful role. In addition medications with endothelial effects such as nebivolol may be useful alternatives to drugs with similar efficacy with side effects.

**References**


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