WHITE PAPER

Endothelial Dysfunction

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Abstract

Endothelial dysfunction is a pathological state that underlies cardiovascular disease, hypertension, and diabetes. Continuing exposure to elevated blood glucose, lipids, homocysteine, toxins, inflammation, and oxidative stress contributes to endothelial damage and atherosclerosis that eventually leads to dysfunction. A deficiency of antioxidants contributes to and accelerates endothelial dysfunction as well.

Damage to the vascular endothelium directly impairs its ability to facilitate vasodilation, regulate blood flow, and safeguard against toxic metabolites. This impairment leads to cardiovascular, endocrine, gastrointestinal, renal, hepatic, and dermatological disorders.

Several biomarker patterns provide clues to early endothelial dysfunction including elevations in homocysteine, glucose, fibrinogen, hs-CRP, ferritin, oxidized LDL, and GGT. Suboptimal omega-3 index, adiponectin, and testosterone can also contribute. A functional blood chemistry analysis of related biomarkers can identify the earliest signs of endothelial dysfunction and provide an opportunity to intervene and resolve them before disease sets in.

Introduction

Cardiovascular disease (CVD) is the primary cause of death worldwide, cutting short an estimated 17.9 lives annually, according to the World Health Organization. Approximately four out of five of those deaths is attributed to heart attack and stroke.[1]

A growing body of evidence indicates that the underlying cause of most CVD is endothelial dysfunction caused by damage to the endothelial lining of the coronary arteries.

We must look at the physiology, function, and dysfunction of the vascular endothelium to understand CVD. We can then intervene to slow the progression of CVD or, preferably, take early steps to prevent it all together.

This review will cover a range of topics related to the endothelium and its role in health and disease including:

- Characteristics of a healthy endothelium
- Detrimental consequences of endothelial dysfunction
- Diseases associated with endothelial dysfunction with a focus on cardiovascular disease
- Nitric oxide, the key to endothelial homeostasis
- Oxidative stress and atherosclerosis
- Signs, symptoms, and diagnosis of endothelial dysfunction
- Biomarkers associated with endothelial dysfunction
- Treatment, intervention, and prevention of endothelial dysfunction

The Endothelium

The endothelium is a thin single-cell layer that forms the inner lining of blood vessels (capillaries, veins, and arteries), as well as the lymphatic system.

This thin layer performs sizable functions including a custodial role in[2] [3] [4]

- Angiogenesis
- Blood fluidity
- Fibrosis
- Immune regulation
- Inflammation
- Modulation of leukocyte adhesion
- Platelet activation and aggregation
- Smooth muscle cell proliferation
- Thrombosis
- Vascular tone and blood flow
- Vasoactive hormone synthesis (e.g., angiotensin II)
- Vasomotor tone (via angiotensinogen, endothelin, nitric oxide, and prostacyclin)
- Wound healing

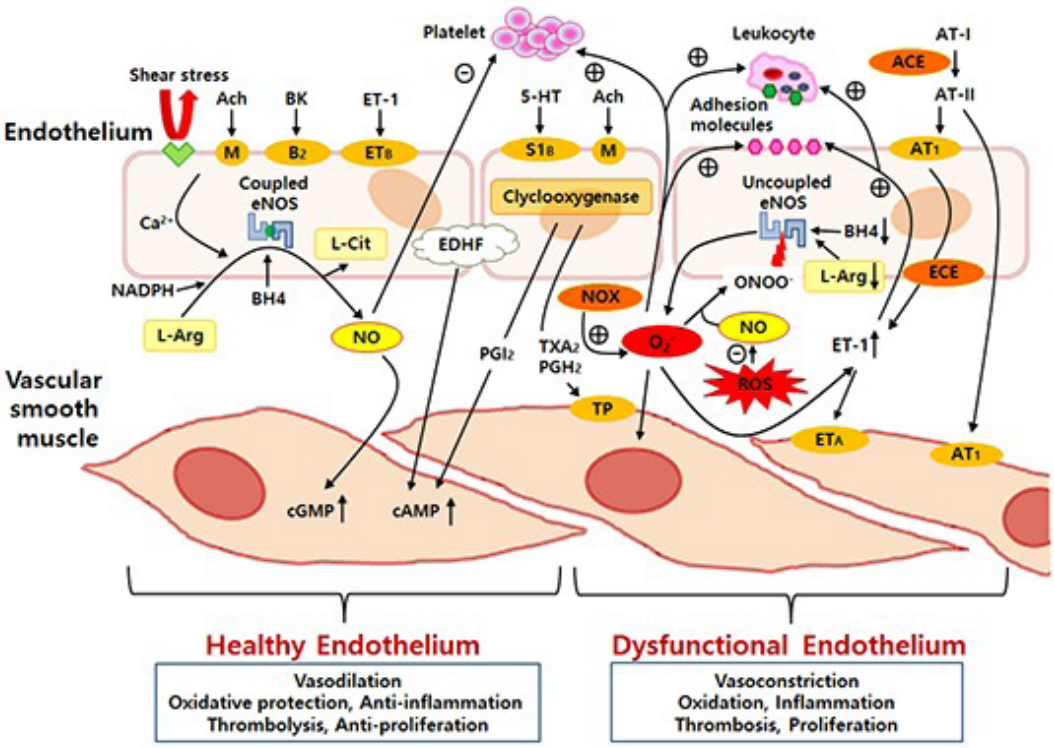
Within blood vessels, the endothelium regulates blood flow by sending relaxation/constriction messages to vascular smooth muscle cells.[5] It also serves as a barrier that protects the smooth muscle, found in the middle of the artery, from toxic metabolites in the blood. When these toxic metabolites, such as homocysteine, chlorine, environmental pollutants, free radicals, and smoking (including e-cigarettes) damage smooth muscle cells, the process of atherosclerosis is initiated.[6] [7] [8] [9] [10]

The protective sentinel of the endothelium is the endothelial glycocalyx, a mesh-like barrier that protects the interior endothelial lining. It comprises membrane-bound endothelial and plasma-derived molecules, including glycoproteins, glycosaminoglycans, and proteoglycans. Hyaluronic acid, thrombomodulin, superoxide-dismutase, and antithrombin III can be found in this dynamic complex layer. Ongoing research suggests the endothelial glycocalyx plays a major role in the homeostasis of the blood vessel wall.[11]

The vascular endothelium can be considered the largest endocrine organ in the body due to its abundant production of vasoactive substances and its balancing act between[12]

- Antioxidation and pro-oxidation
- Growth inhibition and growth promotion
- Anti-thrombosis and pro-thrombosis
- Anti-inflammation and pro-inflammation
- Vasodilation and vasoconstriction
    - Vasodilation is mediated by nitric oxide, endothelium-derived hyperpolarizing factor (EDHF), and prostacyclin.
    - Vasoconstriction is mediated by angiotensin II, endothelin-1 (ET-1), prostaglandin H-2, and thromboxane.[13]

A healthy endothelium is characterized by reduced vascular tension and low oxidative stress… a state maintained with bioactive mediators including nitric oxide (NO). Disruption of this blood vessel bliss by noxious stimuli will cause endothelial dysfunction, laying the groundwork for atherosclerosis and blood vessel damage.



Decreased availability or function of NO in particular contributes to most endothelial dysfunction, and therefore the majority of cardiovascular disease.[14 15 16]

> "An overview of the effects of vascular endothelial factors on the function of vascular smooth muscle and circulating blood cells. In the healthy endothelium, the eNOS is responsible for most of the vascular NO production. However, eNOS becomes a potential ROS generator when in the pathological uncoupled state, due to various oxidative stresses. ACE, angiotensin-converting enzyme; Ach, acetylcholine; AT-I, angiotensin I; AT-II, angiotensin II; AT1, angiotensin 1 receptor; BH4, tetrahydrobiopterin; BK, bradykinin; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; ECE, endothelin converting enzyme; eNOS, endothelial nitric oxide synthase; EDHF, endothelium derived hyperpolarizing factor; ETA and ETB, endothelin A and B receptors; ET-1, endothelin-1; L-Arg, L-arginine; L-Cit, L-citrulline; M, muscarinic receptor; O2-, superoxide anion; ONOO-, peroxynitrite; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; NOX, nicotinamide adenine dinucleotide phosphate oxidase; PGH2, prostaglandin H2; PGI2, prostaglandin I2; ROS, reactive oxygen species; S1B, serotonin receptor; TP, thromboxane prostanoid receptor; TXA2, thromboxane; 5-HT, serotonin; Θ, inhibition; ⊠, stimulation."

Source: Park, Kyoung-Ha, and Woo Jung Park. "Endothelial Dysfunction: Clinical Implications in Cardiovascular Disease and Therapeutic Approaches." Journal of Korean medical science vol. 30,9 (2015): 1213-25. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited

Nitric oxide

Nitric oxide is the keystone to endothelial homeostasis. It is derived from the conditionally essential amino acid arginine through the action of endothelial nitric oxide synthase and the influence of factors such as chemical agonists and shear stress.[17]

Nitric oxide has a protective effect on the endothelium through facilitation of vasodilation, platelet stabilization, inhibition of smooth muscle migration/hyperplasia, and maintenance of an anti-inflammatory milieu.[18]

Specifically, nitric oxide inhibits:[19]

- Inflammation
- Leukocyte adhesion
- Oxidative stress
- Platelet aggregation
- Vascular smooth muscle cell migration and proliferation

However, once the endothelium is damaged, the protective effects of nitric oxide dissipate.[20] The damaged area is very susceptible to fibrosis, lipid deposition, and infiltration of inflammatory immune cells.[21]

Endothelial dysfunction

You will see a pro-inflammatory and pro-thrombotic state with endothelial dysfunction along with progressively reduced vasodilation in affected blood vessels.[22]

Endothelial dysfunction refers to the functional and structural damage that occurs to the endothelium. Endothelial dysfunction and impaired vasodilation are also characteristic of diabetes and hypertension, revealing a "common enemy" among prevalent chronic "diseases."[23]

Diseases associated with endothelial dysfunction

- **Atherosclerosis** - Endothelial dysfunction is damage to the endothelial lining of the artery and is thought to be a key event in the progression to atherosclerosis.
- **Heart attacks and stroke** - endothelial dysfunction has been shown to be of prognostic significance in predicting vascular events including stroke and heart attacks.
- **Diabetes** - Endothelial dysfunction may be a hallmark of hypertension and diabetes[24]
- **Congestive heart failure (CHF)**- Elevated inflammatory markers associated with endothelial dysfunction (CRP, TNF-alpha, von Willebrand factor, fibrinogen) may promote CHF.[25]

Causes of endothelial dysfunction

Traditional and contemporary risk factors for cardiovascular disease and atherosclerosis are found to be directly associated with endothelial dysfunction:[26] [27]

- Adhesion molecule expression
- Arterial hypertension
- BMI (elevated)
- Cigarette smoking
- Diabetes
- Dyslipidemia
- Hyperglycemia
- Hypertension
- Insulin resistance
- Metabolic syndrome
- Oxidative stress
- Oxidized low density lipoprotein
- Proinflammatory cytokines
- Renin-angiotensin axis
- Toxin exposure

Additional promoters of endothelial dysfunction include:

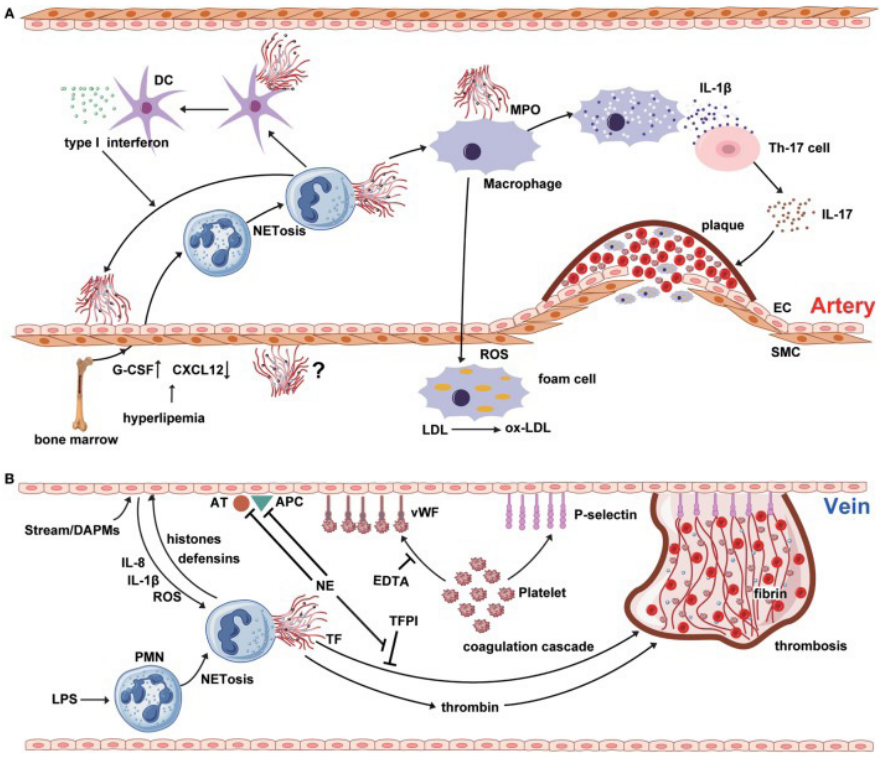
- Poor diet
- Nutrient deficiencies
- Lack of exercise, sedentary lifestyle
- Increased Homocysteine
  - Homocysteine is dangerous because it can induce initial injury to the endothelium, then facilitate the oxidation of the lipid/LDL that accumulates beneath the damaged endothelium. Finally, homocysteine contributes to the abnormal accumulation of blood components around the atherosclerotic lesion.[28] [29]
- **Hyperglycemia**
  - Circulating advanced glycation end-products (AGEs) correlate with atherosclerosis and arterial stiffness in those with coronary artery disease[30]
  - Aortic stiffness was associated with endothelial dysfunction in those with hypertension and diabetes, but not in hypertension without diabetes[31]
  - Endothelial dysfunction may contribute to development of type 2 diabetes and may be present before overt hyperglycemia is detected[32]
    - Endothelial cells are highly metabolic and take up circulating glucose via Glut-1 transporter without the need for insulin.
    - Levels of glucose uptake by endothelial cells reflects circulating glucose levels without any impact from insulin sensitivity.
- **Increased fibrinogen synthesis**
  - Fibrinogen contributes to the clotting process, further jeopardizing the integrity and function of the blood vessel
- **Elevated C-reactive protein (CRP)**
  - CRP is a sign of inflammation and may actually damage the endothelium.[33]
  - C-reactive protein may promote production of growth differentiation factor 15 (CD15), a molecule being researched for its potential as an independent biomarker of cardiovascular disease.[34]

Immune response and endothelial dysfunction

Immune response and infiltration of immune cells can promote endothelial dysfunction and atherosclerosis.

Neutrophils are the most abundant white blood cells of the immune system.[35] These multi-purpose cells can be both protective and destructive. On the downside, their contribution to atherosclerosis has been overlooked. They exude neutrophil extracellular traps (NETs) that trap pathogens but can also trigger endothelial dysfunction, inflammation, and atherosclerosis. NETs have been identified in atherosclerotic plaque.

Researchers suggest that neutrophil counts are significant predictors of cardiovascular events.[36]



NETosis interweaves atherosclerosis and thrombosis.

- Neutrophil extracellular traps (NETs) are involved in the whole process of atherosclerosis. The myeloperoxidase from NTEs can stimulate macrophage to oxidize low-density lipoprotein (LDL) to ox-LDL and form the foam cell. The hyperlipidemia recruits neutrophil into circulation from bone marrow by upregulating the expression of granulocyte colony-stimulating factor and downregulating the level of C-X-C motif ligand -12, which is an important signal for the clearance and recruitment of aged neutrophils to the bone marrow.
- Cholesterol crystals can trigger the polymorphonuclear neutrophil (PMN) to release the NETs that prime the macrophages for pro-inflammatory cytokine production including IL-1β. Then IL-1β activates Th17 cell to release interleukin-17, amplifying the immune cell recruitment into the atherosclerotic plaque.
- As another critical source of foam cell, SMC also takes part in atherosclerosis. However, there are few reports about the interaction between NETs and SMC.
- (B) NETs are released from PMNs, which are activated by LPS or other cytokines from injured endothelial cells.

Oxidative stress contributes to endothelial dysfunction due to:[37] [38]

- Increased reactive oxygen species (ROS) reduce NO bioavailability
- Increased oxidized LDL cholesterol (Ox-LDL)
- Reduced superoxide dismutase (SOD) (SOD can clear ROS)
- Oxidative stress can occur from an excess of oxidative factors and/or an insufficiency of antioxidants
- Intracellular ROS and oxidative stress contribute to the chronic inflammation seen in atherosclerosis[39]
- Hyperlipidemia, hyperglycemia, and hypertension can increase ROS

Oxidative stress and inflammation negatively affect nitric oxide metabolism, as do other factors that disrupt NO and lead to "global vasoconstriction." These factors include mental stress, anger, and cold temperatures.[40]

Reactive oxygen species (ROS) associated with oxidative stress can combine with nitric oxide to produce highly reactive molecules that in turn generate more oxidative stress, more atherosclerosis, and more vascular injury.[41] [42]

Oxidative stress can also reduce availability of nitric oxide by promoting excess degradation of tetrahydrobiopterin. Tetrahydrobiopterin (BH4) is a cofactor for crucial metabolic enzymes including nitric oxide synthase (NOS). It is therefore essential to the maintenance of endothelial function and a healthy blood pressure.

Tetrahydrobiopterin also plays a role in monoamine neurotransmitter synthesis, sensitivity to pain, and immune function. Research suggests that BH4 may be instrumental in restoring NO-redox balance and improving hyperlipidemia, ischemia-reperfusion injury, hypertension, and cardiac hypertrophy.[43] [44] [45]

Administration of BH4 and arginine in subjects with coronary artery disease (CAD) and impaired glucose tolerance/diabetes significantly improved endothelium-dependent vasodilation following ischemia reperfusion.[46]

However, supplemental BH4 may be oxidized to dihydrobiopterin (BH2), posing a challenge to its efficacy.[47]

Atherosclerosis

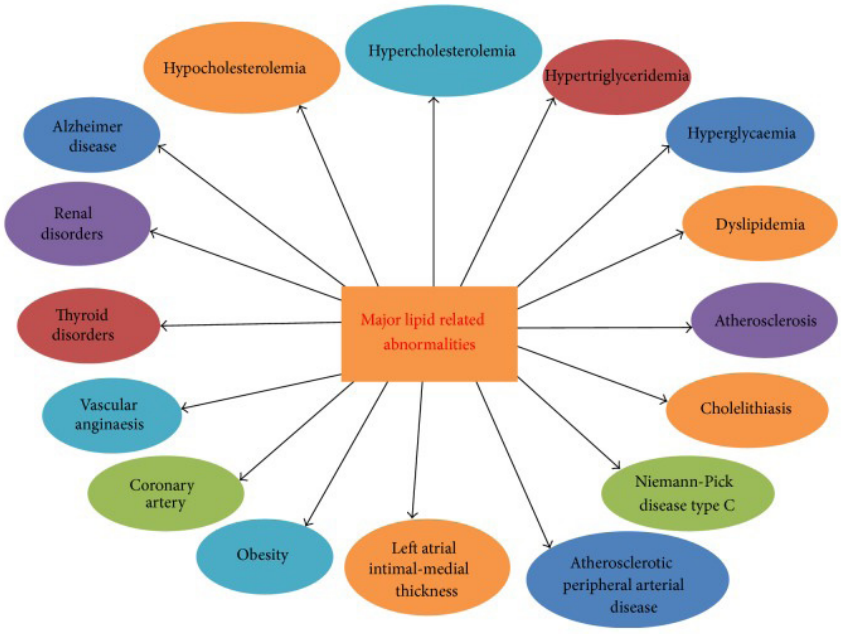
Damage to the endothelium is the first step on the road to full blown atherosclerosis, which contributes to most heart attacks and strokes.[48] Because atherosclerosis causes ischemic heart disease, it is, in fact, considered the main cause of death around the world.

Atherosclerosis is characterized by:[49]

- Endothelial dysfunction
- Vascular inflammation
- Buildup of lipids, cholesterol, calcium, and cellular debris within the intima of the walls of large and medium-size arteries

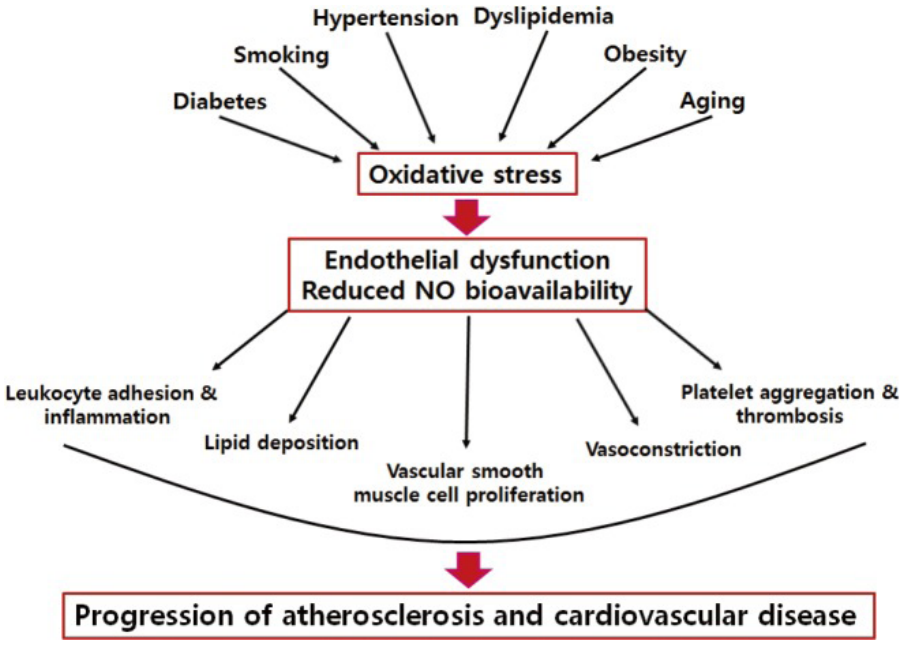
Major risk factors for atherosclerosis include[50]

- Aging
- Antioxidant insufficiency
- Chronic inflammation
- Elevated homocysteine
- Lifestyle factors (sedentary, Western-style diet, obesity, smoking, pollution)
- Oxidative stress



Source: Upadhyay, Ravi Kant. "Emerging risk biomarkers in cardiovascular diseases and disorders." Journal of lipids vol. 2015 (2015): 971453. doi:10.1155/2015/971453 [R] This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Continued exposure to cardiovascular risk factors is mirrored in pathological changes to blood vessels. Loss of integrity of the vascular endothelium is accompanied by atherosclerosis, increased smooth muscle cell migration and proliferation, leukocyte migration, and adhesion.[51]



Progression from risk factors to atherosclerosis and cardiovascular disease mediated by oxidative stress and endothelial dysfunction. The early detection of endothelial dysfunction is a critical point in the prevention of atherosclerosis and cardiovascular disease because this dysfunction could be an initial reversible step in the process of atherosclerosis.

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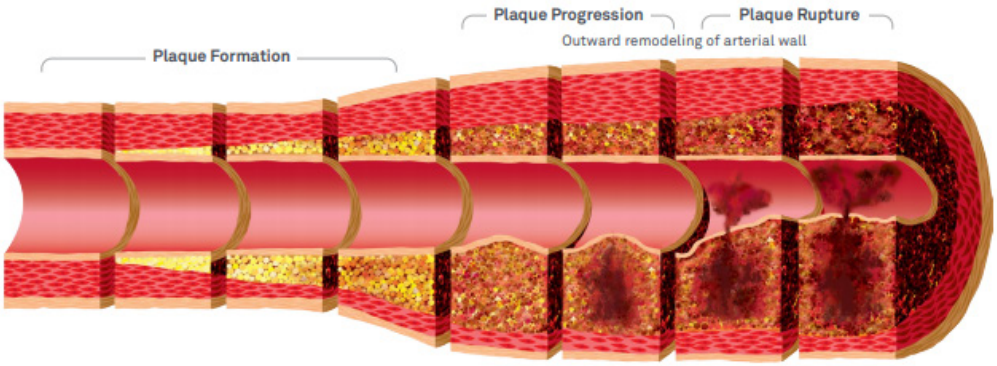
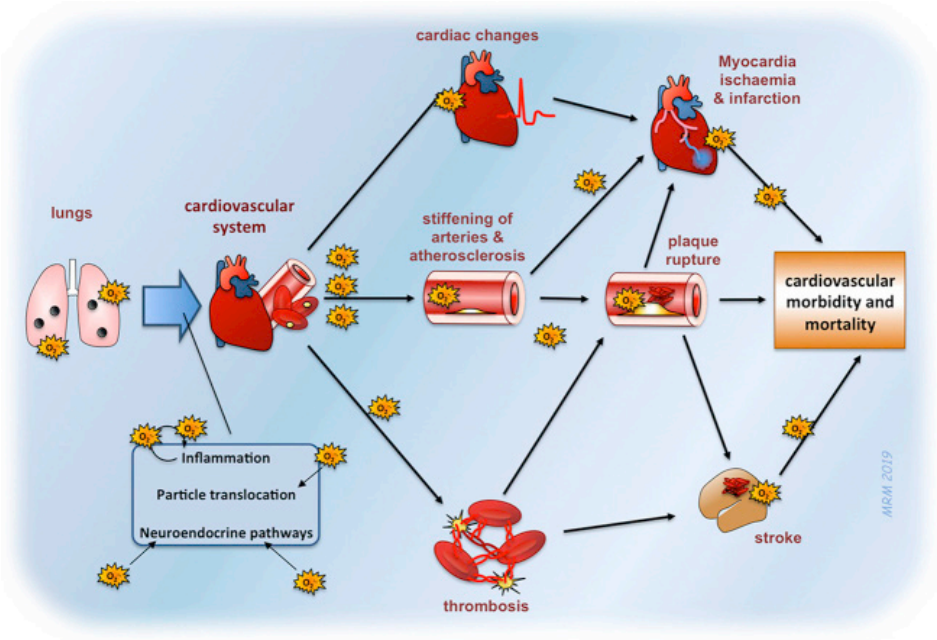


Source: Quest Diagnostics - [R]

Early in vivo research demonstrated that atherosclerotic coronary arteries did not dilate in the presence of vasoactive acetylcholine but constricted instead.[52] Endothelial dysfunction continues its rampage even in advanced atherosclerosis as it can promote the rupture of atherosclerotic plaque.[53]

Summary of endothelial dysfunction, atherosclerosis, and CVD



Source: Miller, Mark R. "Oxidative stress and the cardiovascular effects of air pollution." Free radical biology & medicine vol. 151 (2020): 69-87. doi:10.1016/j.freeradbiomed.2020.01.004 [R] This is an open access article under the CC BY license ([R]).

Pollution and particulate matter contribute to endothelial dysfunction:[54]

- "Endothelial dysfunction is an early initiating event in the vascular disease atherosclerosis. Loss of endothelial function and expression of adhesion molecules attracts and tethers circulating inflammatory cells to the vascular wall.
- Additionally, loss of NO and changes to endothelial cell phenotype encourage the oxidation of circulating lipids (e.g., low density lipoprotein (LDL) to oxidized LDL (oxLDL)) that are

preferentially retained by inflammatory cells that then begin to penetrate the damaged endothelial layer.

- The accumulation of both of inflammatory cells and lipids induces the formation of a fatty plaque in major arteries that grow into the lumen to impede blood flow. Erosion or rupture of advanced plaques is the trigger for thrombosis (a blood clot) that may occlude arteries causing a cardiovascular event such as a heart attack or stroke."

Assessment of Endothelial Dysfunction

Signs and Symptoms

Endothelial dysfunction and disruption are suspect in cardiac disease but may also underlie dermatological, gastrointestinal, hepatic, immunological, renal, and vascular disease. Its presence may be overlooked but its consequences will not go unnoticed.[55]

There may be no overt signs or symptoms of endothelial dysfunction itself and atherosclerosis has a long "silent" asymptomatic phase.[56]

However, in progressing stages of atherosclerosis and cardiovascular endothelial dysfunction, angina can be a telltale sign. Supplementation with 6-9 grams of L-arginine was found to reduce angina and blood pressure and improve endothelial function in CVD patients.[57]

Measuring endothelial dysfunction

Endothelial dysfunction has been recognized as an underlying cause of atherosclerosis and cardiovascular disease for decades... it was first measured in 1986.

Available methods of measuring endothelial dysfunction include[58] [59] [60] [61] [62]

- Doppler flow guide wire
- Intra-arterial infusion of vasoactive agents (e.g. acetylcholine) using coronary angiography, ultrasound
- Flow-mediated dilation of the brachial artery (most common)
- Mercury-filled Silastic strain-gauge plethysmography
- Nitroglycerine-induced vasodilation
- Oscillometric measurement of vascular response
- Reactive- hyperemia-peripheral arterial tonometry (PAT) evaluation of pulse wave amplitude
- Carotid Duplex Ultrasound
- Pulse Wave Velocity (PWV)
- Pressure Pulsation Signal

The noninvasive "cold pressor test" is an interesting test that evaluates sympathetic-mediated release of NO following submersion of a subject's hand in cold water for 2 minutes. Vasoconstriction versus vasodilation will prevail in dysfunctional endothelium. Hyperreactors to the cold stimulus will mount a hypertensive response.[63]

At present, the most convenient and available tool for measuring endothelial dysfunction is flow-mediated dilation (FMD). The test itself is a reflection of nitric oxide production.[64] This method stimulates the brachial artery endothelium to produce vasodilatory factors (e.g., nitric oxide) to help dilate the blood vessel and reduce tension.[65]

The FMD test can be instrumental in predicting cardiovascular events in individuals without overt CVD risk.[66]

Research has identified a group of individuals with endothelial dysfunction who lack commonly recognized cardiovascular risk factors. These individuals are active, maintain a healthy weight, have normal or low blood pressure, and are at low risk for metabolic syndrome.[67]

Therefore, even seemingly healthy individuals with no signs of CVD may be at risk for endothelial dysfunction evidenced by impaired response to vasodilators bradykinin and acetylcholine.[68]

Blood chemistry biomarkers

A number of findings on a blood test can point toward an increasing likelihood of atherosclerosis, inflammation, and endothelial dysfunction:

- **Elevated Homocysteine**
  - Blood levels of homocysteine are associated with endothelial dysfunction:[69]
  - Homocysteine reduces BH4, a cofactor for NO synthesis
  - Conversion of methionine to homocysteine promotes elevated asymmetric dimethylarginine (ADMA) which in turn inhibits endothelial NOS (eNOS)
  - Atherosclerosis increases progressively with a homocysteine level above 11 umol/L.[70]
- **Elevated blood glucose**
  - Post-prandial glucose levels above 122 mg/dL (6.8 mmol/L) may impair flow-mediated arterial dilation.[71]
- **Elevated fibrinogen**
  - Fibrinogen synthesis is stimulated by inflammatory IL-6, promotes coagulation, and increases risk of endothelial dysfunction, CAD, and stroke.[72]
- **Elevated C-reactive protein (CRP, hs-CRP)**
  - Elevation of inflammatory markers such as high sensitivity C-reactive protein may serve as an indirect measure of endothelial function.[73]
  - Hs-CRP was found to be higher when vitamin D was lower in obese non-diabetics free of cardiovascular abnormalities.[74]
- **Insufficient testosterone**
  - Low testosterone (free and total) significantly correlated with reduced flow-mediated vasodilation and was found to be an independent risk factor for endothelial dysfunction in men[75]
- **Elevated iron**
  - High iron levels cause oxidative damage to the endothelial lining and can impair the action of nitric oxide
  - Iron chelation with deferoxamine reduced serum iron significantly from 85±26 to 39±24 µg/dL and improved resting forearm blood flow in both CAD patients and controls. Chelation did not affect ferritin levels which were higher in CAD patients (127±108 ng/mL) than in controls (76±68 ng/mL). Researchers suggest that it may be circulating unbound iron that contributes to lipid peroxidation, NO impairment, and endothelial dysfunction.[76]
- **Elevated ferritin**
  - Ferritin levels increase in inflammation. However, elevated ferritin may also be an

independent risk factor for arterial stiffness and an indicator of the presence of atherosclerosis in those with glucose intolerance.[77]

- **Neutrophil:Lymphocyte Ratio (NLR)**
  - NLR is a marker of systemic inflammation and is associated with increased circulating pro-inflammatory cytokines.
  - NLR can help stratify risk of endothelial dysfunction in asymptomatic patients[78]
    - Low risk in those with neutrophil–lymphocyte ratio <1.5,
    - Intermediate risk in patients between 1.5 and 3,
    - High risk in those with neutrophil- to-lymphocyte ratio >3.
- **Oxidized LDL (OxLDL)**
  - Elevated oxidized low density lipoprotein contributes to foam cell formation, atherosclerosis, and endothelial dysfunction.[79]
  - Risk of atherosclerosis was significantly higher in subjects with levels of oxidized LDL of 44.3 U/L and above. The study concluded that serum oxLDL independently predicted the progression of subclinical atherosclerosis regardless of cholesterol or number and size of LDL particle.[80]
  - Administration of a low dose of oxLDL (8 ug/mL), below that seen in clinical CAD, activated immune mast cells and macrophages and increased monocyte-endothelium adhesion to a greater extent than directly exposing endothelial cell to a higher dose of 80 ug/mL.[81]
- **Elevated asymmetric dimethylarginine (ADMA)**
  - Another potential contributor to endothelial dysfunction is elevated asymmetric dimethylarginine (ADMA)[82]
  - ADMA inhibits synthesis of eNOS and can even cause uncoupling of eNOS which further exacerbates oxidative stress[83]
  - Optimal levels of ADMA are below 100 ng/mL[84]
- **Elevated myeloperoxidase (MPO)**
  - MPO is a pro-oxidant enzyme released by neutrophils and monocytes[85]
    - Reduces nitric oxide bioavailability
    - Increases macrophage uptake of oxidized lipids
    - It may promote atherosclerosis and has been identified in atherosclerotic plaque
    - Levels may help predict outcomes in acute coronary syndromes and in those presenting with chest pain suspected to be due to endothelial dysfunction
- **Elevated malondialdehyde (MDA)[86]**
  - Malondialdehyde is an oxidative stress marker. It is an oxygenated aldehyde produced by the action of free radicals on polyunsaturated fatty acids and cell membrane lipoproteins
  - MDA is elevated by up to 2.48 fold in hyperlipidemia, increasing as level of oxidative stress increased
- **Suboptimal Omega-3 Index**
  - Measures percentage of long-chain omega-3 fatty acids in red blood cell membranes
  - Omega-3 fatty acids have vasoprotective effects[87]
    - Provide antioxidant and anti-inflammatory protection

- Reduce blood pressure
- Improve vasodilation as measured by FMD

  - Optimal goal for omega-3 Index is greater than 8%[88]

- **Elevated Gamma-glutamyl transferase (GGT)**

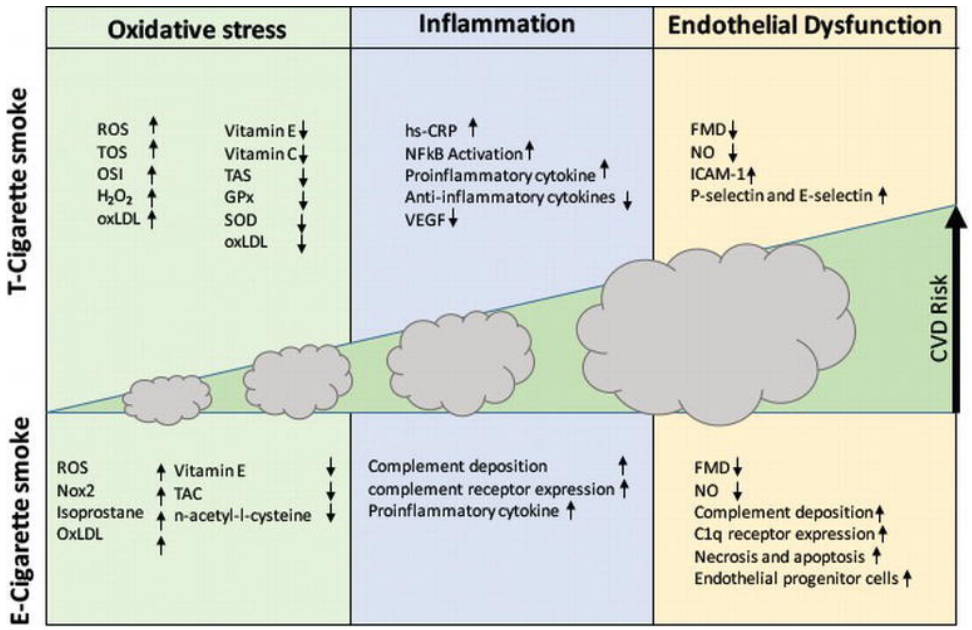
  - GGT can be an important biomarker for atherosclerosis and vascular injury as it is strongly associated with C-reactive protein, oxidized LDL, IL-6, and sICAM-1.[89]
  - Reflects increased glutathione metabolism.
  - Elevated GGT also appears to correlate with cardiovascular and metabolic disorders including congestive heart failure, vascular events, type 2 diabetes, hypertension.[90]
  - GGT may be an independent risk factor for cardiovascular disease and events. In healthy males free of heart disease, those with the highest GGT (35 units/L or greater) had a 2.34 greater risk of acute coronary event than those with a GGT of less than 13 units/L.[91]

- **Low adiponectin**

  - Adiponectin is an anti-inflammatory cytokine released by adipose tissue.
  - Factors that increase serum levels of adiponectin include aging, calorie restriction, and estrogen deficiency, and factors that decrease it include oxidative stress, cigarette smoke, obesity and type 2 diabetes.[92]
  - Low levels of adiponectin were found to be associated with endothelial dysfunction in diabetics and non-diabetics. In non-diabetics, low adiponectin was the only predictor of endothelial dysfunction compared to HOMA-IR, BMI, triglycerides, and insulin. Serum adiponectin has been found to be inversely correlated with carotid intima-thickness in both healthy and diabetic individuals.[93]

- **Inflammation from cigarette smoking**

  - Not surprisingly, cigarette smokers had significantly increased levels of hs-CRP and WBCs (total WBCs, basophils, lymphocytes, monocytes, neutrophils). Smokers also tend to have increased levels of pro-inflammatory IL-6 and decreased levels of anti-inflammatory IL-10.[94]



Advanced biomarkers in endothelial dysfunction[95] [96]

- Elevated cellular adhesion molecules (released from damaged endothelial cells)
- Vascular cell adhesion molecule-1 – VCAM-1, endothelial leukocyte adhesion molecule-1 E-selectin, intercellular adhesion molecule-1-ICAM-1
- Elevated von Willebrand factor indicative of endothelial damage
- Elevated Endothelin-1 peptide
- Soluble NOX2-derived peptide (sNOX2-dp) and 8-iso-prostaglandin F2α (8-isoPGF2α) reflect oxidative stress.
- Endothelial progenitor cells (EPCs) and microvesicles (MVs) are biomarkers for inflammation and endothelial dysfunction.
    - Elevated endothelial progenitor cells from bone marrow assist in endothelial repair

Treatments

Allopathic approaches to endothelial dysfunction are based on its identification once established versus early prevention.

- A number of pharmaceutical interventions have been researched including eNOS enhancers, nitrate therapy, alpha-beta blockers for blood pressure management, calcium channel blockers, ACE inhibitors to treat high blood pressure and heart failure, statins, novel therapies (e.g. ranolazine, aminophylline).[97] [98] Interestingly, it is the antioxidant function of some of these pharmaceuticals that protect the endothelium.
- Bradykinin and acetylcholine can be administered to stimulate NO synthesis, while nitroglycerine and sodium nitroprusside can be converted to nitric oxide.[99]
- External counter pulsation (ECP) therapy may be used to help supply oxygen-rich blood to the heart.[100]
- The NSAID indomethacin (as well as vitamin C) was found to reverse the blunted vasodilation response to acetylcholine by restoring bioavailability of nitric oxide.

Functional naturopathic approach

Functional approaches to endothelial dysfunction rely on recognition of contributing factors and early assessment of related biomarkers.

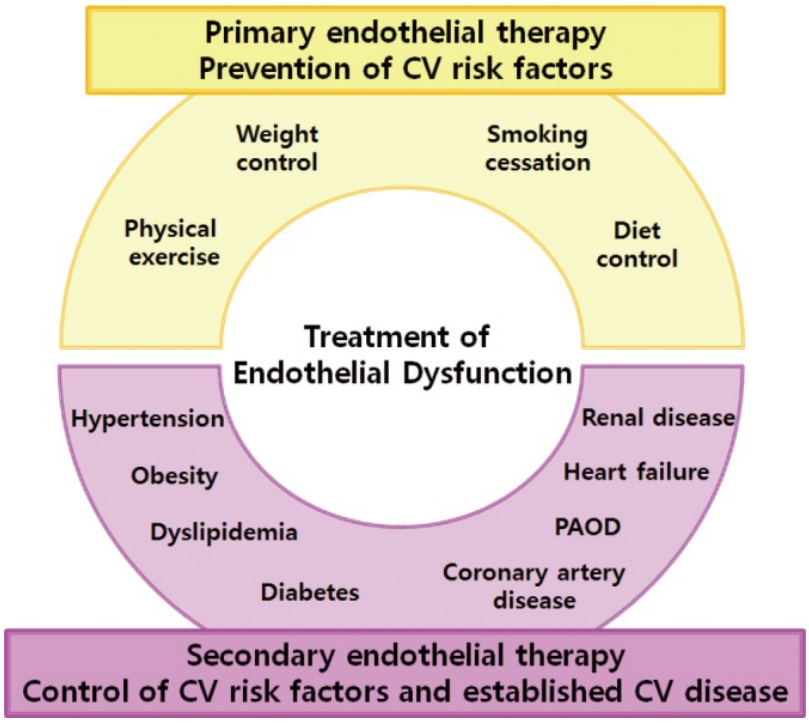
Early stages of endothelial dysfunction may be reversible prior to full progression to atherosclerosis.[101]

Major modifiable factors contributing to endothelial dysfunction include unhealthy diet (inflammatory, processed foods, low in micronutrients, antioxidant, phytonutrients, fiber, omega-3s, monounsaturated fats); exposure to cigarette smoke, toxins, and pollution; excess inflammatory compounds and oxidative stress; nutrient insufficiencies; and sedentary lifestyle. Assessing these factors should be the first step in evaluating risk of atherosclerosis and endothelial dysfunction.

Remember, unbridled oxidative stress and inflammation disrupt nitric oxide metabolism and promote endothelial dysfunction. They must be addressed to order to rein in this potentially debilitating condition:[102]

It is likely that excess inflammation from any source, even periodontitis or arthritis, can cause damage to the vascular endothelium so a full history is essential.[103]

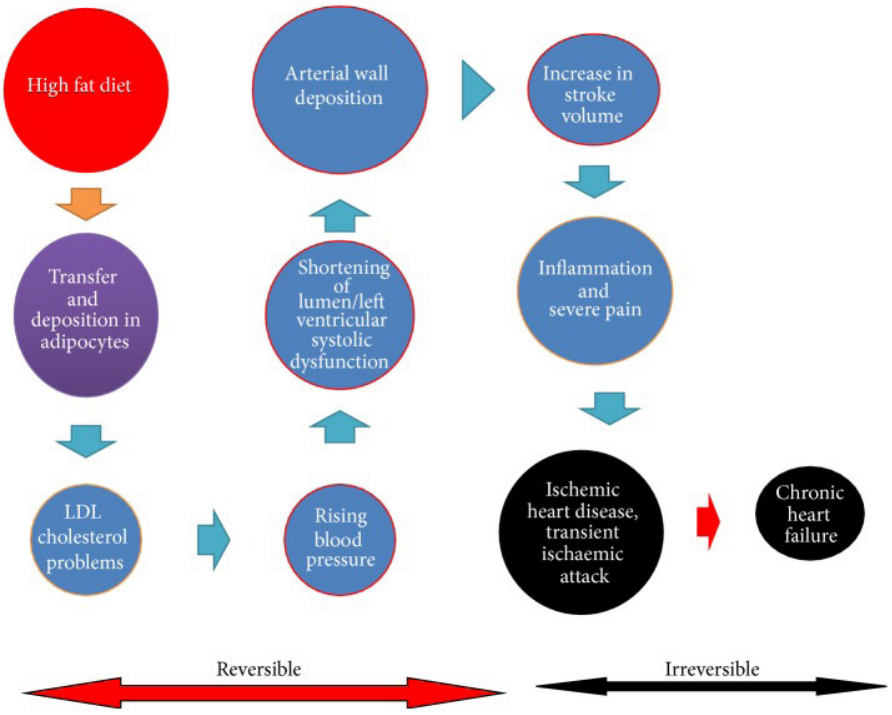
The functional, naturopathic approach to endothelial dysfunction is recognition of its causes and mitigation of their effects. Early recognition of oxidative stress risk and detection of endothelial dysfunction is critical to reversing or preventing atherosclerosis and its progression to CVD.[104]



Therapeutic approaches to endothelial dysfunction. Endothelial therapy can be achieved with primary endothelial therapy for prevention of healthy endothelial function by controlling cardiovascular risk factors and secondary endothelial therapy to improve dysfunctional endothelial homeostasis by treating underlying cardiovascular risk factors and cardiovascular disease. CV, cardiovascular; PAOD, peripheral arterial occlusive disease.[105]

Factors associated with endothelial dysfunction	Interventions that improve endothelial function
Increased age	L-arginine
Male sex	Antioxidants
Family history of CHD	Smoking cessation
Smoking	Cholesterol lowering
Increased serum cholesterol	ACE inhibitors
Low serum HDL-cholesterol	Exercise
Hypertension	Mediterranean Diet
Increased serum homocysteine	
Diabetes mellitus	
Obesity	
High-fat meal	

Source: Widmer, R Jay, and Amir Lerman. "Endothelial dysfunction and cardiovascular disease." Global cardiology science & practice vol. 2014,3 291-308. 16 Oct. 2014, doi:10.5339/gcsp.2014.43 [R] This is an open access article distributed under the terms of the Creative Commons Attribution license CC BY 4.0, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.



Successive progression of transient ischemic attack and chronic heart failure in man.

Source: Upadhyay, Ravi Kant. "Emerging risk biomarkers in cardiovascular diseases and disorders." Journal of lipids vol. 2015 (2015): 971453. doi:10.1155/2015/971453 [R] This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Negative effects of a high-fat diet

Meta-analysis indicates that endothelial function is typically compromised in adults following a high-fat mixed meal and that the observed impairment in brachial artery flow-mediated dilation is a sign of cardiovascular risk, morbidity and mortality. Researchers investigated whether adolescents would demonstrate similar changes in post-prandial flow mediated dilation.

A small study of 10 adolescents observed that a high-fat meal also high in protein did not significantly impair arterial dilation in the same way a high-fat diet was expected to. Also, addition of insoluble wheat fiber to a high-fat meal blunted post-prandial hypertriglyceridemia and related changes in flow-mediated arterial dilation. Researchers recommend conducting larger studies to investigate these relationships further.[106]

A high-fat Western-style diet can impair endothelial function for up to four hours. To investigate mitigation of this threat, a randomized, double-blind, placebo-controlled study of phytonutrient supplementation in 38 healthy adult volunteers was conducted.

Results indicated that daily supplementation with fruit and vegetable concentrates, including those enhanced with antioxidant nutrients, significantly improved flow-mediated vasodilation using the brachial artery reactivity test (BART) following a high-fat fast food meal containing 50 grams of fat.

In the supplement group, serum total and LDL cholesterol decreased significantly, and serum levels of nitric oxide metabolites increased significantly.[107]

This study followed up the observation that supplementation with antioxidants vitamin C and vitamin E prevented the significant reduction in vasodilation that occurs following a high-fat meal.[108]

A healthy plant-based diet and healthy lifestyle are preventive

Phytonutrients/phytochemicals are compounds unique to plant-based foods. Many of them have antioxidant and anti-inflammatory benefits.

Abundant intake of fruits, vegetables, phytonutrients, and antioxidants is closely associated with optimal health, including reduction of cardiovascular risk factors. Research demonstrates that phytonutrients and antioxidants can exert positive effects on endothelial function, including its generation of nitric oxide. These positive effects are not new news, they just seem to have been forgotten in the medical models that have been developed.

Omega-3 fatty acids can have a vasoprotective effect in endothelial dysfunction as demonstrated in a number of studies in those with metabolic syndrome, elevated BMI, hyperlipidemia, and those who smoked cigarettes. A benefit of omega-3 intake in in diabetics was also observed in two of five studies reviewed. Specific vasoprotective effects of omega-3 fatty acids include:[109]

- Anti-inflammatory activity
- Decreased blood pressure
- Improved vasodilation as measured by FMD
- Increased antioxidant protection

Naturally occurring antioxidants can have important protective effects against endothelial dysfunction: [110]

N-acetylcysteine (NAC)

- Has potent antioxidant effects
- Essential to synthesis of glutathione
- Inhibits inflammatory cytokine release, NADPH oxidase expression, and white blood cell adhesion
- Improves endothelial response with and without presence of atherosclerosis

Vitamin C

- Scavenges superoxide, therefore preventing lipid peroxidation, platelet/neutrophil activation, adhesion molecule upregulation, and scavenging of NO
- Scavenges reactive nitrogen species, inhibits LDL oxidation
- Can improve endothelium response in diabetes, hypercholesterolemia, hypertension, smoking
- The lowest measured tertile of vitamin C in a study of type 1 diabetics 0.25 – 0.86 mg/dL (14.02-49.01 umol/L) versus the highest tertile 1.48-2.10 mg/dL (84.01 to 118.99 umol/L) was significantly associated with greater carotid intima-media thickness, a presumed sign of atherosclerosis.[111]
- Administration of vitamin C restored nitric oxide bioavailability and reversed a blunted vasodilation response to acetylcholine.[112]

Vitamin E

- Scavenges hydroperoxyl radicals as a lipid-soluble antioxidant
- Protects endothelial function in hypercholesterolemia and smoking

Supplementation

Adherence to a healthy diet is essential to addressing atherosclerosis and endothelial dysfunction. However, supplementation may be beneficial as well, especially considering that micronutrient deficiencies are common among the general population but even more common in those with hypertension, cardiovascular disease, environmental toxin exposure, and prescription drug use.[113]

Nutrition supplementation can significantly improve endothelial function as evidenced by a number of clinical studies.

Vitamin C

Vitamin C (ascorbate/ascorbic acid) is of particular interest due to its potent antioxidant activity as a free radical scavenger and the fact that humans cannot synthesize ascorbate while most mammals can.

- Vitamin C is required for the function and optimal permeability of the endothelium and its insufficiency is noted as a cause of endothelial dysfunction.[114]
- A small prospective study indicated that daily antioxidant supplementation with 1 gram of vitamin C and 500 mg of vitamin E significantly improve endothelial function as evidenced by restoration of flow-mediated dilation. Beneficial effects disappeared 1 month after supplementation ceased.[115]
- Vitamin C (1 gram/day) and vitamin E (440 IU/day) significantly improved FMD in adult male hypertensive patients as evidenced in a randomized double-blind study.[116]
- Supplementation with vitamin C (500 mg/day) and vitamin E (400 IU/day) also significantly improved endothelial function and FMD in a randomized double-blind study of children with hyperlipidemia 9-20 years of age.[117]

Arginine

Arginine supplementation may also be of specific benefit in endothelial dysfunction.

- Administration of arginine was found to improve endothelial dysfunction in subjects with high cholesterol and atherosclerosis.[118] [119]
- L- arginine, applied intravenously or via intracoronary administration improved endothelium-dependent vasodilation in such patients. The D-arginine form is not effective.[120]
- Intervention with arginine and vitamins B6, B12, and folic acid significantly improved vascular function in 40 individuals with moderately elevated blood pressure. Homocysteine and systolic blood pressure were significantly reduced in this randomized double-blind study as well.[121]

A variety of nutrients are found to have antihypertensive effects, therefore reducing stress and further damage to the vascular endothelium. These include[122]

Alpha lipoic acid	Monounsaturated fats	Taurine
Calcium	N-acetylcysteine	Vitamin B6
CoQ10	Omega-3 fatty acids	Vitamin C
Flavonoids	alpha linolenic acid, EPA,	Vitamin D
Gamma-linolenic acid	DHA	Vitamin E, gamma/delta
Garlic	Pomegranate	tocopherols, tocotrienols
L-carnitine	Potassium	Zinc
Magnesium	Pycnogenol	
Melatonin	Resveratrol	

Coenzyme Q10

Coenzyme Q10 is produced endogenously though its production is impaired by HMG-CoA reductase inhibitors (statin drugs). Supplementation with 150 mg of CoQ10 per day can support antioxidant function, reduce inflammatory IL-6, and reduce oxidative stress in those with coronary artery disease.[123]

A randomized, double-blind, controlled study of 20 individuals with advanced atherosclerosis and CAD indicated that supplementation with 10 mg of melatonin was associated with a significant decrease in mean levels of ICAM, VCAM, and CRP, and a significant increase in nitric oxide.[124]

Vitamin B12

Preliminary research suggests a role for cobalamin as a cofactor in the metabolism of nitric oxide. Researchers propose that the intermediate glutathionyl-cobalamin is an active form of B12 that participates in production and function of nitric oxide and in turn affects cell membrane protection, and immune and vascular health.[125]

Pomegranate

A meta-analysis of 16 studies indicated that supplementation with pomegranate juice had a significant effect on inflammatory markers hs-CRP, IL-6, and TNF-alpha.[126]

Dark Chocolate

Dark chocolate (high in flavonoids and antioxidant factors) significantly improved endothelial-dependent coronary vascular function, reduced oxidative stress, and reduced platelet adhesion in 22 heart transplant patients.[127]

Physical Activity

Though a topic unto itself, physical activity and exercise can improve endothelial function… no surprise there![128]

Animal and human research support the application of regular, robust physical activity to support cardiovascular and endothelial function. A minimum of 40 minutes of physical activity at least 3 days per week is recommended. Optimal activity includes 10,000 steps per day and engagement in aerobic activity 3 days per week.[129]

Optimal Takeaways

- Hypertension, diabetes, obesity, hyperlipidemia, and smoking are risk factors for thrombosis, atherosclerosis, and endothelial dysfunction.[130]
- Combined with oxidative stress and a pro-inflammatory milieu, these risk factors form the main pillars underlying cardiovascular risk.
- Major modifiable factors include diet, activity, lifestyle, nutrient insufficiencies, supplementation, exposure to toxins and pollutants, and stress management.
- Failure to address these factors will propel individuals down the road from metabolic disturbance to chronic, life-threatening disease.
- Basically, a healthy lifestyle makes for a healthy life.
- Allopathic treatments are based on identification of endothelial dysfunction once it occurs instead of early preventative measures.
- Functional naturopathic approaches to endothelial dysfunction rely on recognizing and addressing contributing factors and associated biomarkers.

Early intervention and prevention of endothelial dysfunction includes:

- An optimal flow-mediated dilation (FMD) reading, a reflection of nitric oxide production
- A healthy diet rich in fresh fruits and vegetables, antioxidants, omega-3 fatty acids, monounsaturated fats, anti-inflammatory herbs and spices, vitamins, minerals, and phytonutrients
- Targeted nutrition supplementation
- Minimization of exposure to toxins, pollution, cigarette smoke, and stress
- Regular robust physical activity
- Maintain a desirable body weight and lean body mass
- Stress management
- Address biomarkers out of the optimal range including those related to oxidative stress, inflammation, and blood glucose regulation:

Elevated

- Homocysteine
- Blood glucose
- Fibrinogen
- C-reactive protein (CRP, hs-CRP)
- Iron levels
- Neutrophil:Lymphocyte Ratio (NLR)
- Oxidized LDL (OxLDL)
- Asymmetric dimethylarginine (ADMA)
- Myeloperoxidase (MPO)
- Malondialdehyde (MDA)
- Gamma-glutamyl transferase (GGT)

Decreased

- Omega-3 Index
- Adiponectin
- Testosterone

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