The Role of Chronic Inflammation in Cardiovascular Disease and its Regulation by Nutrients

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Abstract

Multiple risk markers for atherosclerosis and cardiovascular disease act in a synergistic way through inflammatory pathways. This article discusses some of the key inflammatory biochemical risk markers for cardiovascular disease; in particular, the role of three basic cell types affected by these risk markers (endothelial cells, smooth muscle cells, and immune cells), the crucial role of inflammatory mediators, nitric oxide balance in cardiovascular pathology, and the use of nutrients to circumvent several of these inflammatory pathways.

Most risk markers for cardiovascular disease have a pro-inflammatory component, which stimulates the release of a number of active molecules such as inflammatory mediators, reactive oxygen species, nitric oxide, and peroxynitrite from endothelial, vascular smooth muscle, and immune cells in response to injury. Nitric oxide plays a pivotal role in preventing the progression of atherosclerosis through its ability to induce vasodilation, suppress vascular smooth muscle proliferation, and reduce vascular lesion formation. Nutrients such as arginine, antioxidants (vitamins C and E, lipoic acid, glutathione), and enzyme cofactors (vitamins B2 and B3, folate, and tetrahydrobiopterin) help to elevate nitric oxide levels and may play an important role in the management of cardiovascular disease. Other dietary components such as DHA/EPA from fish oil, tocotrienols, vitamins B6 and B12, and quercetin contribute further to mitigating the inflammatory process.

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Introduction

Multiple risk factors for atherosclerosis and cardiovascular disease include disordered lipid profiles, autoimmunity, infection, homocysteine, asymmetrical dimethylarginine, C-reactive protein, genetic predisposition, and various metabolic diseases.[1-5] Many risk factors act in a coordinated or synergistic way through one or two inflammatory pathways. Risk factors appear to act on three cell types that coordinate their action to influence cardiovascular dynamics, function, and structure. These cell types include:

- Endothelial cells that line the vascular lumen. They control the intra- and transcellular flow of nutrients, hormones, and immune cells, and regulate vascular tone and blood flow.[6]
- Smooth muscle cells (SMC) or vascular smooth muscle cells (VSMC) that maintain vascular tone and structure.
- Immune cells, including monocytes/macrophages and T lymphocytes, which defend the endothelium and SMC from chemical and biological insult.

The disruption or over-expression of the coordinated activities of these cells can lead to cardiovascular disease.[7-10] Chronic inflammation

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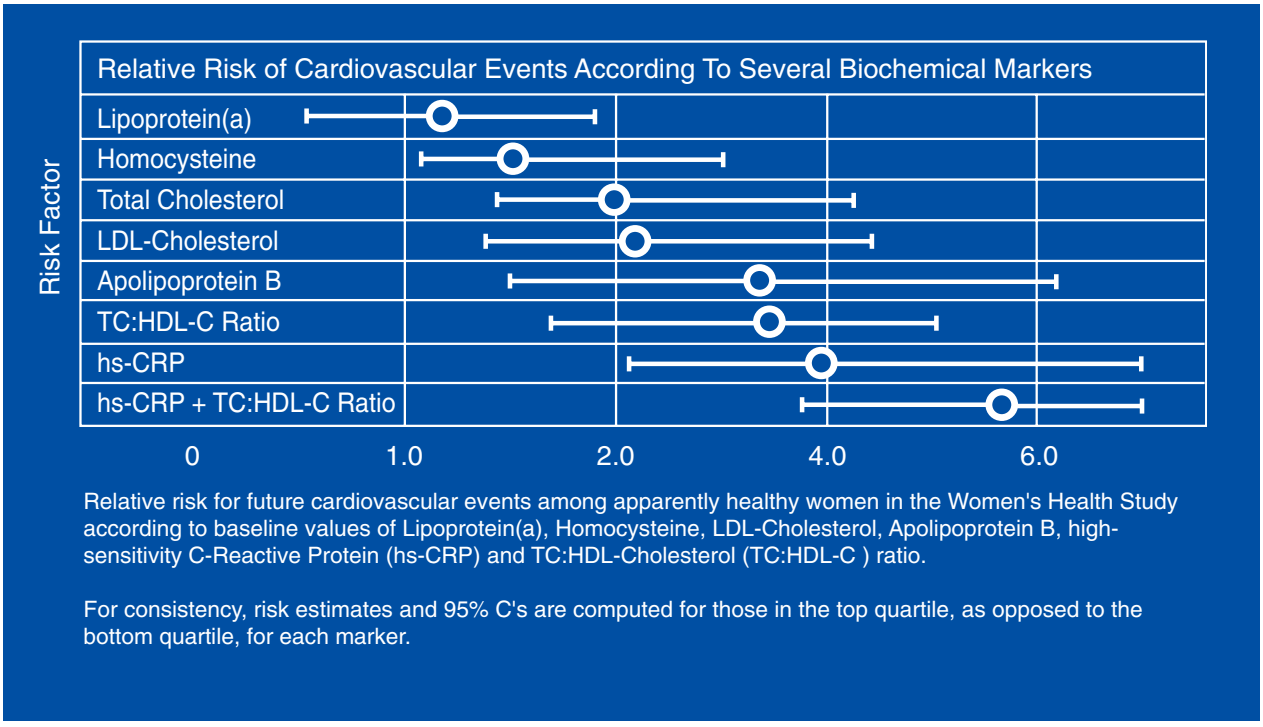
Figure 1. *Relative and Synergistic Risk among Several Associated Factors*



Relative risk for future cardiovascular events among apparently healthy women in the Women's Health Study according to baseline values of Lipoprotein(a), Homocysteine, LDL-Cholesterol, Apolipoprotein B, high-sensitivity C-Reactive Protein (hs-CRP) and TC:HDL-Cholesterol (TC:HDL-C) ratio.

For consistency, risk estimates and 95% C's are computed for those in the top quartile, as opposed to the bottom quartile, for each marker.

is the most common disruptor of the activities of these cells. Risk factors for cardiovascular disease that have a pro-inflammatory component include LDL cholesterol, smoking, elevated blood sugar, hypertension, diabetes, infection, homocysteine, ischemia, oxidant damage, interleukin-6, lipoprotein (a), high sensitivity C-reactive protein (hs-CRP), serum intracellular adhesion molecule-1, and apolipoprotein-B.[2,4,5,11-14] In addition, these inflammatory risk markers can react synergistically to increase relative risk (Figure 1). One common link among these risk factors is the activity and metabolism of nitric oxide (NO).

Endothelial Cell Function

Endothelial cells play a vital physiological role in dividing blood from tissue. These cells actively inhibit the activation of the hemostatic mechanism and maintain blood circulation and fluidity, limit the efflux of cells and protein from the bloodstream, and participate in the maintenance of normal vasomotor tone.[6]

Endothelial cells are highly metabolically active and behave in a similar manner to paracrine or endocrine gland cells in the release of chemical mediators.[10,15,16] The endothelium generates a number of active molecules in response to injury or toxic chemical or oxidant stimuli, such as:

- Adhesion molecules, intracellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1), fibronectin, selectins, interleukin-1, heparin sulfate[17]
- Clotting or coagulation factors[17,18] (von Willebrand Factor, thromboxane, prostacyclin)
- Fibrinolysis factors[19,20] (e.g., tissue plasminogen factor)

- Components of the renin-angiotensin system[21] (e.g., angiotensin II that acts as a pro-inflammatory cytokine and augments the production of reactive oxygen species)
- Prostaglandins[22-24] (e.g., prostacyclin)
- Growth-promoting or angiogenesis factors (transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF))[25]
- Vascular tone regulators[18,25-28] (NO and endothelin-1)

These biological molecules demonstrate that the endothelium senses change in the local milieu, and respond by releasing a variety of cytokines and chemicals that regulate vascular smooth muscle relaxation/contraction, vascular structure, platelet and monocyte function, and coagulation.[26,28,31]

The endothelium secretes a number of vascular-relaxing substances as well as several vasoconstricting agents (Table 1). However, one of the most potent endogenous vasodilators is endothelial-derived nitric oxide. NO is a critical modulator of blood flow and blood pressure,[27,32] and opposes the vasoconstricting effects of endothelin, angiotensin II, serotonin, and norepinephrine.[31.33,34] NO also suppresses the proliferation of vascular smooth muscle.[28,35]

It was initially thought a continuous basal synthesis of NO from the vascular endothelium maintained resting vascular tone. Recent evidence, however, suggests that NO production is increased whenever the endothelium is damaged or stressed; otherwise, only residual synthesis occurs.[36] Deficiency or loss of NO activity contributes not only to increased vascular resistance but to blood vessel medial thickening and/or myointimal hyperplasia, thus altering the structure of the vascular bed (Table 2).[27,35,37]

A second messenger of internal cellular communication – cyclic GMP (cGMP), produced in response to nitric oxide – is a key regulator of vascular smooth muscle cell contractility, growth, and differentiation.[40] It is implicated in opposing the pathophysiology of hypertension, cardiac hypertrophy, atherosclerosis, and vascular injury/ restenosis (Figure 2).[43,46]

Function of Vascular Smooth Muscle Cells

Vascular smooth muscle cells contribute to the maintenance of vascular tone. The balance between stimuli that initiate contraction or dilation is important in providing the elastic recoil essential for normal functioning of the arteries.[33] Contraction of vascular smooth muscle (VSM) can be initiated by mechanical, electrical, and chemical stimuli. Passive stretching of VSM can cause contraction that originates from the smooth muscle itself. A number of stimuli such as norepinephrine, angiotensin II, vasopressin, endothelin-1, and thromboxane (TXA_2) can elicit contraction.[33] Each of these substances binds to specific receptors on the VSMC or onto endothelial receptors adjacent to VSM and causes contraction of smooth muscle.

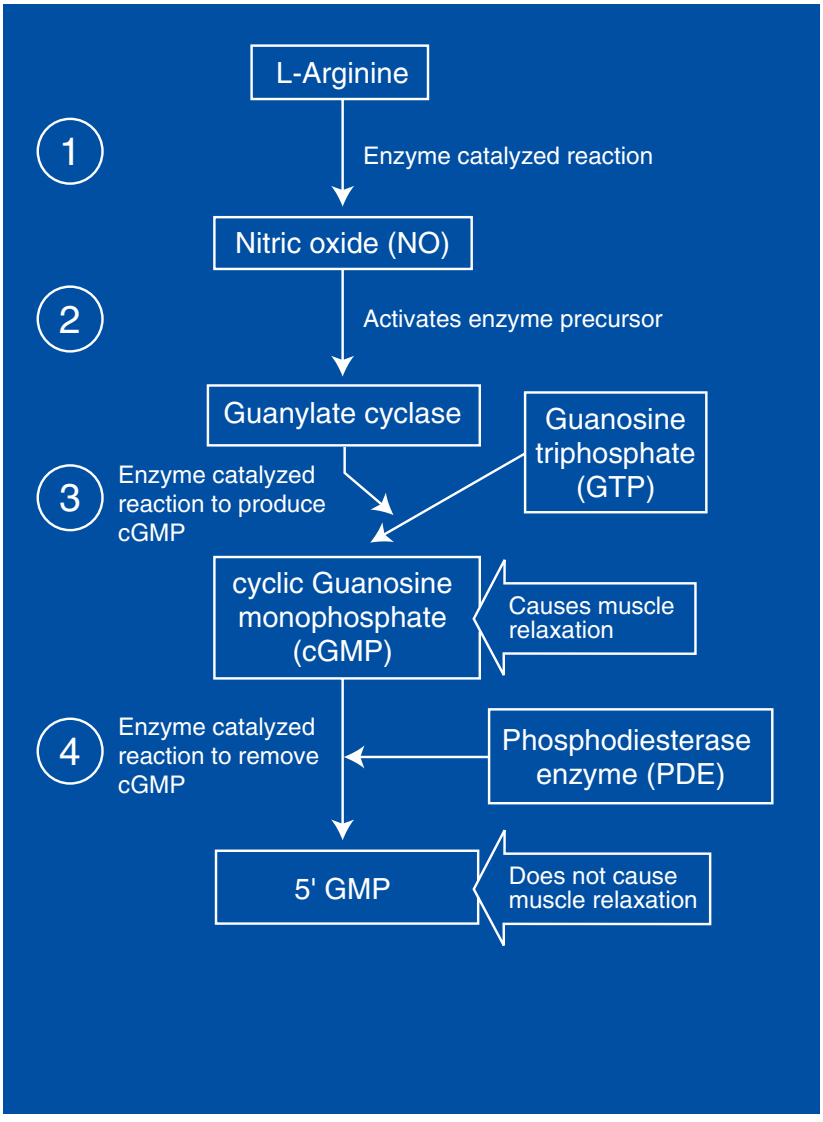
Nitric oxide, epinephrine, and prostacyclin can induce vasodilation of vascular smooth muscle.[28,47] NO is synthesized by a constitutive form of nitric oxide synthase (NOS) located in the endothelial lining of blood vessels and is a major contributor to regulation of blood pressure and blood flow. However, during hypertension and in atherogenesis, SMC change phenotype from an elastic mode to a secretory mode.[48-50] These activated VSMC secrete and release a range of growth promoters and chemo-attractants.[51-53] This phenotypic change is crucial to the mechanical strength of the atheromatous plaque.[54] Proliferating SMC can secrete matrix proteins and thicken the vascular wall. If these proteins are rich in collagen and elastic fibrils, the structural strength of the atheroma is assured, as a rich matrix of collagen forms a solid cap over the vascular lesion.[54-56]

Lesions, however, that develop and increase in size exhibit increased cholesterol/lipid deposits and show signs of increased cell death (particularly SMC death).[54,55] SMC can undergo apoptosis, weakening the vascular wall and causing aneurisms (Table 3).[57,58] The result is a lesion

Table 1. The Balance between Contracting and Dilating Factors

Endothelial-derived Relaxing Factors	
Prostacyclin (PGI2)	Decreases platelet adhesion and aggregation, as well as promoting relaxation of vascular smooth muscle. It inhibits endothelin-1 release.[22-24]
Adrenomedullin (AM)	A potent vasodilator peptide that protects the vascular system from oxidative stress.[44]
Endothelial-derived Hyperpolarizing Factor (EDHF)	It hyperpolarizes VSM by stimulating the cellular membrane potassium/calcium pump, thereby preventing smooth muscle contraction. It is activated by shear pressure associated with blood flow.[22-24]
C-type Natriuretic Peptide (CNP)	Also known as endothelium-derived factor, is a vascular dilator. It also inhibits growth and proliferation of vascular smooth muscle.[25]
Nitric Oxide (NO)	A soluble gas that diffuses through water and lipid phases, it is a potent vasodilator. It is derived from the amino acid arginine through the action of the enzyme, nitric oxide synthase (NOS).[29] Its production is influenced by a number of factors: shear pressure (i.e., hemodynamic shear stress exerted by viscous drag of flowing blood) and various bioactive molecules such as estrogen, acetylcholine, bradykinin, substance P, histamine, insulin, bacterial endotoxins, adenosine, and thromboxane.[23]
Endothelial-derived Contracting Factors	
Endothelins (ET)	There are a number of isoforms of endothelins (ET-1, ET-2, and ET-3) with a wide range of biological actions. ET-1 is a potent vasoconstrictor and pressor agent. It is released by the endothelium. ET-1 release is stimulated by angiotensin II, antidiuretic hormone, thrombin, cytokines, and reactive oxygen species. Its release is inhibited by NO, prostacyclin, and atrial natriuretic peptide.[23,24,30]
Thromboxane (TXA2)	Activates its own receptor on the VSMC and causes vasoconstriction.[23]
Prostaglandin H2	Activates thromboxane receptors.[23]
Angiotensin II	Is a potent vasoconstrictor and pressor agent. It is produced by the action of angiotensin-converting enzyme on angiotensin1.[24,31]
Superoxide Anion ($O_2^{\cdot-}$)	Quenches NO, thus contributing to vasoconstrictor tone. It can produce vasoconstriction in its own right. It is produced during infection, inflammation, or high oxidant stress.[23]

***Figure 2**. The Biochemical Pathway of NO Activation of cGMP*



with a large lipid pool that may weaken and rupture, allowing lipid or atheroma fragments to enter the circulation.[59] After rupture, exposure of the underlying lesion (collagen fragments) to the blood vessel initiates thrombotic episodes of platelet and thrombin aggregation that may lead to organ failure or tissue damage through embolus. Small ruptures of atheroma plaque frequently reseal, incorporating thrombi into the lesion.[54,60,61]

As this sequence of events persists, the plaque increases in bulk, incorporating platelets, which further stimulates cell proliferation through the release of platelet-derived growth factor (PDGF). If the rupture is massive, this may lead to prothrombotic stimuli sufficient to occlude the lumen of the blood vessel.[56,60]

The VSM accumulation seen around an atheroma can be viewed as a beneficial repair process. Failure to repair through inhibition of cell proliferation or stimulation of apoptosis may reduce VSM accumulation, which can be detrimental as it increases the risk of plaque rupture.

The Contribution of Immune Cells – Monocytes/Macrophages and T Lymphocytes

In atherosclerosis, macrophages are important for intracellular lipid accumulation and foam cell formation. Monocytes respond to chemotactic factors (monocyte chemo-attractant protein MCP-1), cytokines, and macrophage growth factors produced by vascular endothelial cells, smooth muscle cells, and infiltrated cells, by migrating from peripheral blood into the arterial intima and differentiating into macrophages. Unquenched intracellular reactive oxygen species (ROS) induce monocytes to differentiate into macrophages.[62] Macrophages express a variety of receptors, particularly scavenger receptors, and take up modified lipoproteins, including oxidized low-density lipoprotein, beta-very-low-density lipoprotein, and/or enzymatically degraded low-density lipoprotein. These cells accumulate cholesterol esters in the cytoplasm, which leads to foam cell formation in lesion development. In addition, macrophages and macrophage-derived

Table 2. *Activity of NO*

NO Actions	NO Deficiency
Induces vasodilatation.[29,81]	Impairs endothelial vasodilatation.[23,38,45]
Reduces blood pressure.[27,32,38]	Increases vascular resistance.[27,38]
Suppresses proliferation of vascular smooth muscle.[28,29,35]	Contributes to vascular medial thickening and/or myointimal hyperplasia.
Reduces lesion formation after vascular injury.[29]	Accelerates vascular lesions by increasing platelet aggregation and immune cell migration to the lesion.
Inhibits interaction of circulating immune cells with the vascular wall by inhibiting adhesion molecule activation and expression. Prevents platelet aggregation or thrombus formation.[27,29]	Contributes to abnormal vasomotor tone and ischemic conditions.[102]
Prevents the progression of atherosclerosis.	Contributes to the initiation and progression of atherosclerosis.[143]
Induces or activates guanylate cyclase, thus increasing cellular cGMP (Figure 2) in SMC and inducing muscle relaxation.[23]	
Disrupts free radical and oxidant-mediated reactions. Binds with super oxide anion.[23,39]	Increases oxidant stress and vascular injury. Excess superoxide anion binds with NO to form peroxynitrite.[39,41,42]

foam cells produce ceroid and advanced glycated end-products (AGEs) and accumulate these substances in their cytoplasm. Extracellularly generated AGEs are taken up by macrophages via receptors for AGEs. Most foam cells die in loco because of apoptosis and some foam cells escape from the lesions into peripheral blood. Macrophages also play multifaceted roles in inducing plaque rupture, blood coagulation, and fibrinolysis via the production of various enzymes, activators, inhibitors, and bioactive mediators. During the development of atherosclerosis, macrophages interact with vascular endothelial cells, medial smooth muscle cells, and infiltrated inflammatory cells, particularly T cells and dendritic cells.[63,64]

Activation of endothelial cells causes blood monocytes and T lymphocytes to stick to the luminal surface of the endothelium. Monocytes squeeze through the junction between the endothelial cells and enter the sub-endothelium, which is between the endothelium and the internal elastic lamina. Normally, the single endothelial layer lies almost directly over the internal elastic lamina. However, in the initial development of an atheromatous lesion, monocytes/macrophages fill this potential space.[54,64] Oxidized lipids/cholesterol that

Table 3. *Properties of SMC in Advanced Plaques*[54-58,60]

1. Poor proliferation
2. Early senescence
3. Increased apoptosis (programmed cell death)
4. Increased cellular DNA damage in VSMC
5. Increased sensitivity to oxidized lipids/cholesterol and peroxynitrite, resulting in induced plaque; VSMC death while leaving normal VSMC in the artery unaffected
6. Inflammatory cells adjacent to the plaque can kill plaque VSMC.
7. Apoptotic VSMC release pro-inflammatory cytokines and membrane bound micro-particles into the circulation, which can initiate a pro-coagulant cascade as well as recruiting monocytes and macrophages to the surrounding area.

may be present in the lesion are scavenged by macrophages, as they form toxic foam cells. In human atherosclerotic lesions, many of the macrophage foam cells also contain ceroid – an insoluble polymer formed by oxidation of mixtures of lipid and protein.[63] Figure 3 summarizes the interactions of monocytes/macrophages with modified/oxidized LDL.

Further recruitment of monocytes and macrophages can occur by the release of cytokines from the endothelium and VSM as part of the inflammatory cycle.[54,63,64] These cells attempt to remove apoptotic cell debris, although the presence of modified or oxidized LDL may hamper this debris removal,[65,66] resulting in the recruitment of more inflammatory cells and the subsequent release of Fas-L (a death-inducing ligand) and death of surrounding or adjacent neutrophils, monocytes, and activated VSMC.[58,67] As a result, the atheromatous plaque core becomes rich in macrophages as the plaque ages.

Once activated, macrophages can overexpress the production of matrix-degrading enzymes (matrix metalloproteins; MMPs) and prothrombin.[68] This process also activates SMC and increases the production of excessive ROS that induce oxidative modification of LDLs.[69,70] A vicious cycle ensues of endothelial cell activation or dysfunction that induces the expression of VCAM-1 and monocyte chemo-attractant proteins (MCP-1), leading to increased monocyte/macrophage recruitment into the intima.[64]

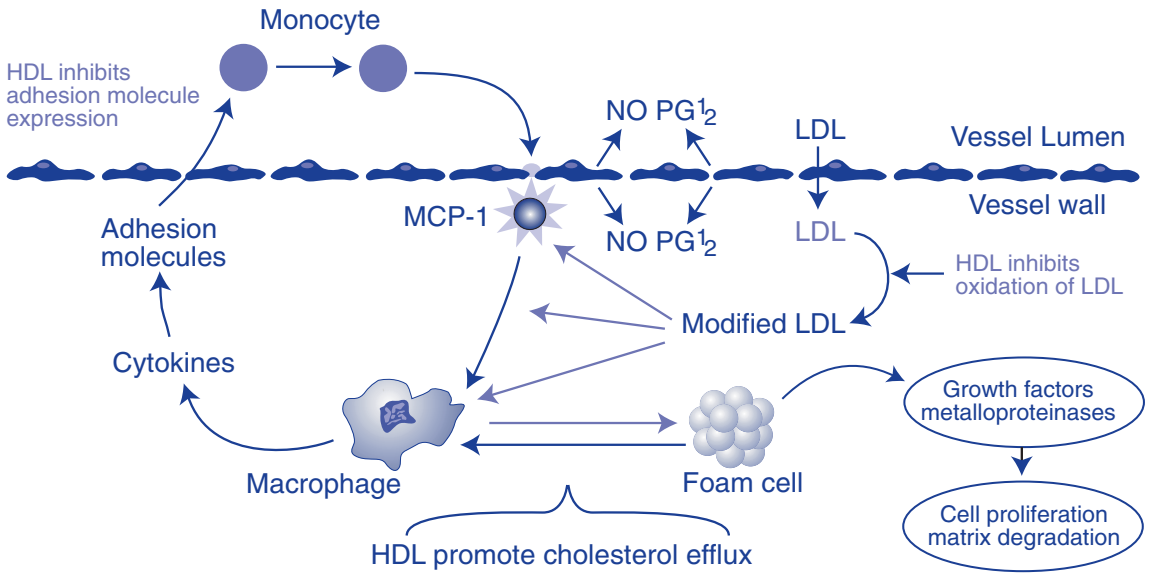
Oxidative stress also decreases the expression of endothelial nitric oxide synthase (eNOS) by endothelial cells.[71] As eNOS limits monocyte/macrophage-endothelial cell interaction, the loss of eNOS or its decreased expression results in formation of a macrophage-rich atheroma. This results in a soft plaque that increases the risk of unstable angina, thrombosis, and acute myocardial infarction.[72,73]

Macrophages and T lymphocytes can also produce NO through an inducible nitric oxide synthase mechanism (iNOS).[41,74] The excess NO can react with the superoxide anion to produce peroxynitrite, a very aggressive free radical species that can induce cellular apoptosis, cellular mitochondrial dysfunction, and lipid peroxidation.[41,42]

Three basic isoforms of NOS enzymes have been identified that generate NO from the amino acid arginine:[41,74-76]

- Endothelial nitric oxide synthase (eNOS) – a constitutive NOS which is Ca++/calmodulin-dependent[77,78]
- Neuronal isoforms (nNOS), which are the normal constituents of healthy cells and neurons
- Inducible isoforms (iNOS), which are not normally expressed by vascular tissue but by immune cells

Figure 3. *Monocyte/Macrophage and Foam Cell Formation*



Inducible NOS is calcium-independent and is stimulated by cytokines such as interferon-gamma and interleukin-1β.[41,77,78] iNOS-derived NO plays an important role in numerous physiological and pathophysiological conditions (e.g., blood pressure regulation, inflammation, and infection).[79,80]

eNOS and nNOS generate NO, but NO generation from these two isoforms can have opposing roles in the process of ischemic injury. While increased NO production from nNOS in neurons can cause neuronal injury, endothelial NO production from eNOS can decrease ischemic injury by inducing vasodilation.[76]

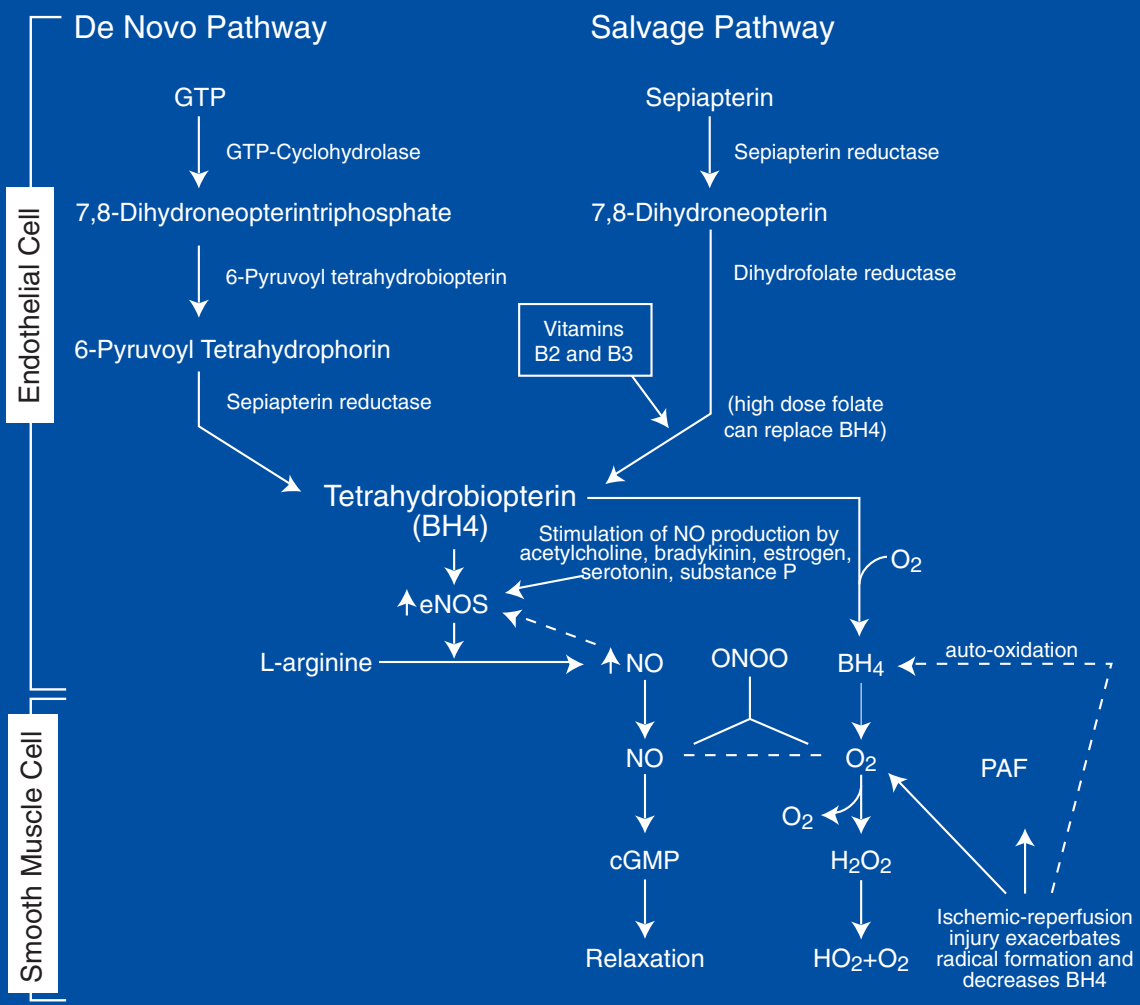
Nitric Oxide: Its Clinical Relevance

Many studies suggest NO is a potent, endogenous anti-atherogenic molecule that suppresses key processes in atherosclerosis (Table 2).[29,39,81] As mentioned previously, nitric oxide is produced through the action of the enzyme nitric oxide synthase on the amino acid arginine to produce nitric oxide and citrulline.[23,82,83] The cofactors required for this reaction include vitamin B3 (a cofactor for nicotinamide adenine dinucleotide phosphate),[41,84] vitamin B2 (a cofactor for flavin adenine dinucleotide),[41,84] tetrahydrobiopterin (BH4),[77,84,85] and calmodulin (a calcium-ion modulator).[41,84]

Tetrahydrobiopterin stabilizes NO synthase and facilitates the binding to L-arginine (Figure 4). Under conditions when intracellular concentration of tetrahydrobiopterin is reduced, NO synthase generates superoxide anions instead of NO.[84] Under physiological conditions there is a balance between endothelial production of NO and oxygen-derived free radicals.

Once synthesized, NO diffuses across the endothelial cell membrane and enters the vascular smooth muscle cells where it activates the production of the second cellular system cGMP (Figure 2).[23] Once activated, this messenger system plays numerous roles such as controlling vascular tone and platelet and mitochondrial function.[27,86]

Figure 4. *The Role of Tetrahydrobiopterin in Production of NO*[41,84,85]



Tetrahydrobiopterin (BH4) acts as a cofactor for the action of NOS activity. BH4 is susceptible to auto-oxidation with the resultant production of superoxide radicals. The superoxide anion produced can react with NO to produce peroxynitrite (ONOO-) and thus reduce NO activity. Superoxide anion is reduced to hydrogen peroxide (H2O2) by the enzyme superoxide dismutase, which is a copper- or selenium-dependent enzyme.
Adapted from: M Viljoen et al. Geneeskunde, The Medicine Journal 2001;43(8)

Decreased production of NO, or decreased sensitivity to the action of NO, has consistently been shown to impair endothelial-dependent vasodilation, contributing to the pathogenesis of atherogenesis.[22,23,31,34,71] Many risk factors interfere with or are associated with endothelium-dependent vasodilation, including hyperlipidemia, hypertension, types 1 and 2 diabetes, cigarette smoking, hyperhomocysteinemia, infection, inflammation, low birth weight, insulin resistance,

hypercholesterolemia, chronic kidney disease, microalbuminuria, AGEs, age-related vascular changes, and a family history of heart disease.[5,6,86-91]

Excessive production of NO can also contribute to vascular cell pathology, as excessive NO can disrupt mitochondrial function and ATP production,[92] indirectly initiate apoptosis,[93] and lead to formation of the peroxynitrite radical and other cytotoxic substances.[42,94] These negative effects may be due to timing of release, duration of action, and concentration of NO at a particular cellular point as well as the oxidative state within its area of activity. [95]

Mechanisms Involved in Decreased Nitric Oxide Levels

Deficiency in Cofactor Vitamins B3 and B2 and Tetrahydrobiopterin (BH4)

A decreased intake of the cofactor or an increased requirement of BH4, due to, for example, diabetes, smoking, or hypercholesterolemia, may cause cofactor deficiencies.[89] In addition, oxidant stress increases BH4 destruction.[71] In either case, deficiency of these cofactors, whether a relative demand deficiency or local tissue deficiency, can result in decreased NO production and impaired endothelial vasodilation. In clinical situations, abnormalities in BH4 metabolism have been implicated in the endothelial dysfunction observed in hypertension, reperfusion injury, homocysteinemia, hypercholesterolemia, and smoking.[71,96,97] Vitamin C and folic acid are important in stabilizing and maintaining intracellular levels of BH4.[98-100]

Decreased or Increased Nitric Oxide Synthase Enzyme Expression and Activity

Hyperglycemia causes increased eNOS expression with a concomitant increase in superoxide anion production, resulting in NO inactivation.[39] Chronic inflammation or bacterial endotoxins can increase the synthesis of iNOS and induce hypotension by excessive production of NO.[41,79,80] In advanced atherosclerosis, reduced expression of eNOS enzyme has been observed, possibly due to the action of oxidized LDLs.[35,101]

Increased Endogenous Nitric Oxide Synthase Inhibitors

Two of the most potent endogenous inhibitors of NOS are asymmetric dimethylarginine (ADMA) and symmetrical dimethylarginine (SDMA).[102,103] These two endogenous inhibitors are synthesized from methylated arginine-rich proteins.[102,104] ADMA is further metabolized to citrulline and methylamines by the action of the enzyme dimethylarginine dimethylaminohydrolase (DDAH), of which two isoforms (DDAH I and II) have been identified.[103-105] Therefore, inhibition or modulation of DDAH will have a profound effect on plasma ADMA levels. Oxidized LDL cholesterol, hyperglycemia, and oxidant stress can cause a decline in DDAH activity.[35,36,106-108]

Elevated levels of endogenous ADMA are predictive of vascular lesion formation.[103,105,109] Plasma elevation of ADMA has been observed in the following disease states: chronic renal failure,[105,110] hypercholesterolemia,[105] congestive heart failure,[105,110] hypertension,[111] atherosclerosis,[105] homocysteinemia,[112,113] Raynauds disease,[114] and in situations resulting in oxidative stress[102] – tobacco smoking, aging, diabetes, and insulin resistance.[35,106-108,115,116] These are the common risk factors associated with atherosclerosis and coronary artery diseases.

The administration of L-arginine and vitamin E has been shown to improve endothelium-dependent vascular function in subjects with high ADMA levels.[110,117,118]

Decreased Nitric Oxide Bioavailability

Nitric oxide can react with superoxide anions to produce peroxynitrite anions, thus quenching the biological effects of NO.[39] In conditions associated with oxidative stress, such as hypercholesterolemia and glucocorticoid excess, NO production may be high but inactivated, resulting in impairment of endothelial-dependent

vasodilation.[38,39,119] Quenching free radicals with lipoic acid,[120-124] coenzyme Q10,[125] quercetin,[128.129] vitamins C and E,[130-132] superoxide dismutase,[131] and glutathione[133-138] results in the reduction of NO degradation and maintenance of endothelial function.[88,139]

Decreased Vascular Smooth Muscle Sensitivity to Nitric Oxide

Diabetes and hyperglycemia-induced hypo-responsiveness in vascular smooth muscle may be overcome by increasing the activity of guanylate cyclase, the enzyme that increases the synthesis of cGMP, the second cellular messenger system stimulated by NO.[140]

Furthermore, this impaired vasodilation in response to NO derived from vascular endothelium or organic nitrates in vascular smooth muscle may be related to increased degradation of the second messenger cyclic guanosine monophosphate by type 5 phosphodiesterase.[40]

Several common cardiovascular risk factors or disease states impair nitric oxide synthesis as well as its activity.[22,71,141-143] Therefore, it is not surprising that NO is a major player in cardiovascular physiology.

Increasing Levels of Nitric Oxide

Fortunately, some of the risk factors noted above can be managed by increasing the synthesis and activity of NO by:

- Supplementing with arginine (as it has been shown to compete with ADMA) to prevent the inhibition of eNOS by this endogenous inhibitor. It normalizes endothelial vasodilation in hypercholesterolemic/hypertensive, and hyperhomocysteinemic patients.[97,116]

- Supplementing with antioxidants to reduce the oxidative stress strongly implicated in endothelial dysfunction. Vitamins C and E, lipoic acid, glutathione, and superoxide dismutase can increase the bioavailability of NO, reduce oxidative stress, and increase DDAH activity.[35,144,145]

- Ensuring nutrient cofactors – vitamins B2 and B3 and tetrahydrobiopterin – are available to activate NOS. High-dose folic acid can be a substitute for tetrahydrobiopterin.[81,96,99,116]

Auxiliary Nutrients to Reduce Cardiovascular Risk

The most important factor determining plaque stability is the plasma level of atherogenic LDL particles.[146] Increased levels of these particles cause endothelial dysfunction with impaired vasodilation capacity and heightened vasoconstriction, as well as inducing and maintaining inflammatory infiltration of the plaque, impairing the strength of the fibrous cap, and facilitating aggregation and coagulation.[146]

Lipid-lowering treatments (e.g., tocotrienols,[147-149] and supplemental DHA/EPA and omega-3 rich diets[150-153]) can decrease the risk of plaque rupture and subsequent thrombogenicity, as well as normalize the impaired endothelial function in hypercholesterolemic patients.[154]

Furthermore, lipid lowering diminishes inflammation and macrophage accumulation, as well as increases interstitial collagen accumulation in atheroma, resulting in an increase in a plaque's mechanical stability.[112,155] Thus, a decrease in lipid levels, along with modification of other risk factors, has the potential to become a cornerstone for treatment of acute coronary syndromes, in addition to being an effective treatment in primary and secondary prevention of coronary heart disease.[146]

The presence of oxidized LDL in atherosclerotic lesions supports the contention that oxidant stress is a contributing factor to atherosclerosis.[156-158] As a corollary, antioxidants that can inhibit LDL oxidation may be regarded as antiatherogenic. This concept is supported by animal studies showing that antioxidants such as probucol, butylated hydroxytoluene, tocotrienols, and alpha-tocopherol can slow the progression of atherosclerosis.[147-149,158] Epidemiological and clinical data indicate a protective role of dietary antioxidants against cardiovascular disease, including vitamin E, beta-carotene, and vitamin C.[159-164] Likewise,

basic research studies on LDL oxidation have demonstrated a protective role for antioxidants, present either in the aqueous environment of LDL or associated with the lipoprotein itself.[158]

Quercetin has been shown to be inversely associated with mortality from coronary heart disease[159,165,166] by inhibiting the expression of metalloproteinase 1 (MMP-1), thus inhibiting the disruption of atherosclerotic plaques and contributing to plaque stabilization.

Lipoic acid plays a crucial role in preventing atherosclerosis. It induces the production of NO and inhibits the activation of monocyte chemo-attractant protein-1.[120,144,167-169] It also improves NO-mediated vasodilation in diabetic patients.[170,171]

Hyperhomocysteinemia is an inflammatory risk factor for cardiovascular disease for which nutritional supplementation is indicated.[100,172] High levels of homocysteine induce sustained injury of arterial endothelial cells and proliferation of arterial smooth muscle cells, and enhance expression/activity of key participants in vascular inflammation, atherogenesis, and vulnerability of the established atherosclerotic plaque.[173] Other effects of homocysteine include impaired generation and decreased bioavailability of NO, interference with transcription factors and signal transduction, oxidation of LDLs, and decreased endothelium-dependent vasodilation.[173]

Reduction of homocysteine by vitamins B6 and B12 and folate is crucial in reducing cardiovascular risk and oxidant stress associated with elevated plasma levels.[172,173] Folate reduces plasma homocysteine levels and enhances eNO synthesis and shows anti-inflammatory activity.[100] It stimulates endogenous BH4 (a cofactor necessary for eNO synthesis). BH4, in turn, enhances NO generation and augments arginine transport into the cells. Folic acid increases the concentration of omega-3 PUFAs, which also enhance eNO synthesis.[100] Vitamin C augments eNO synthesis by increasing intracellular BH4 and stabilization of BH4.[98,99] The ability of folate to augment eNO generation is independent of its capacity to lower plasma homocysteine levels.[100]

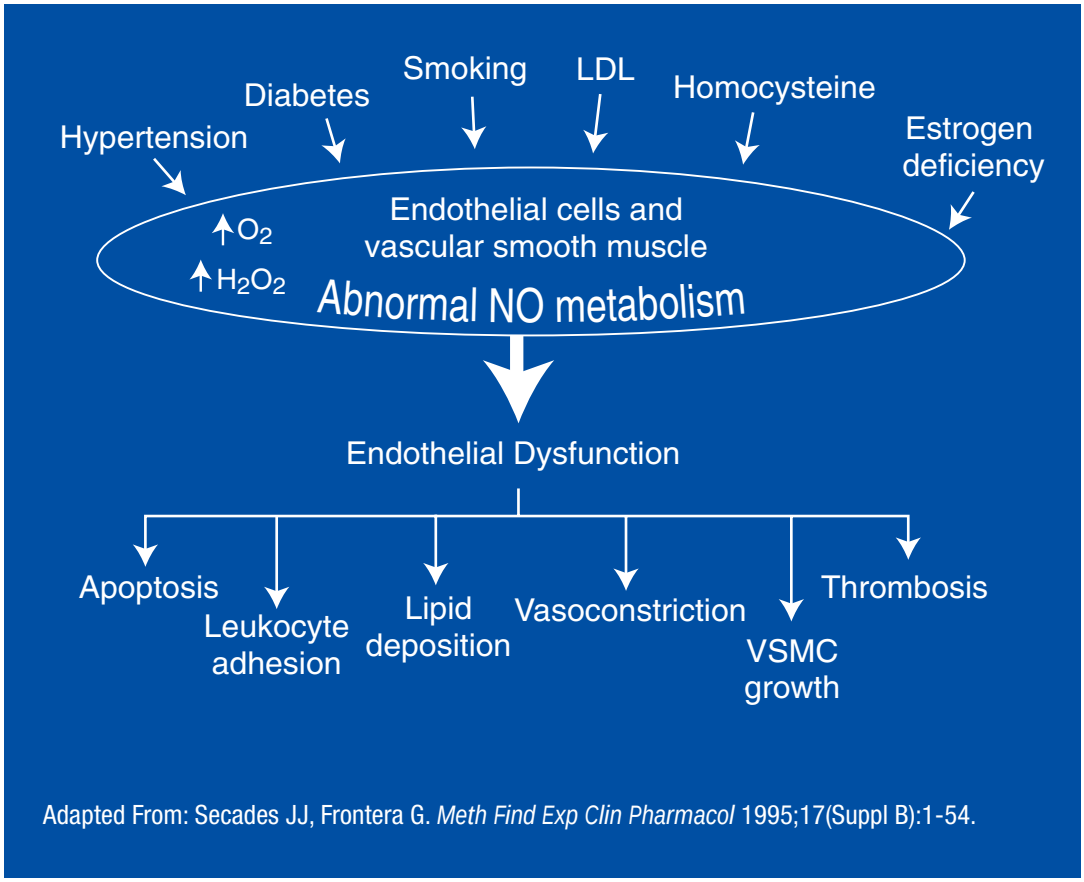
Table 4. *The effects of ROS on Endothelium and VSMC*

Reactive Oxygen Species[63]

- Impair vascular function by injuring endothelial and VSMC membranes
- React with NO, activating it[23]
- Oxidize tetrahydrobiopterin, the cofactor for NOS
- Peroxidize low density lipoproteins (LDL) to oxidized LDLs, which in turn upregulates adhesion molecules on endothelial cells and PDGF receptors on SMC, resulting in SMC proliferation and extracellular matrix synthesis
- Stimulate the synthesis of asymmetric dimethylarginine (ADMA), inhibiting NOS activity or expression[71]
- Inhibit guanylate cyclase, leading to a decrease in cGMP, which decreases the action of NO on SMC
- In the vasculature promote the expression of receptors and chemotactic agents to facilitate the migration of inflammatory cells to the development of an atheroma[172]

ROS are generated within the vessel wall by several mechanisms, including a vascular type of a NAD (P) H oxidase.[126] Mechanical stress, environmental factors, cytokines, low-density lipoproteins (LDL), and exposure to catalytic metal ions can stimulate ROS formation. Their ability to modify LDL, react with endothelial-derived nitric oxide subsequently forming peroxynitrite, and to amplify the expression of various genes important for leucocyte recruitment within the arterial wall are the basis of the oxidant injury theory of atherosclerosis. In animal studies, antioxidant therapy (probucol, butylated hydroxytoluene, N', N'-diphenylenediamide, vitamin E, superoxide dismutase) have been successfully used to prevent fatty streak formation, and to restore impaired nitric oxide-dependent vaso relaxation.[127]

Figure 5. *Common Risk Factors, Oxidative Stress, and Endothelial Dysfunction*



Adapted From: Secades JJ, Frontera G. *Meth Find Exp Clin Pharmacol* 1995;17(Suppl B):1-54.

Discussion

From a physiological point of view, the major contributors to atherosclerotic plaque formation include macrophage accumulation, smooth muscle cell activation, endothelial cell activation, oxidative stress giving rise to altered blood rheology and vascular tone, and plaque build-up. This process leads to basically two forms of plaque – stable and unstable. Unstable plaques are characterized by a thin fibrous cap overlying a macrophage/lipid-rich core, while stable fibrous plaques have a solid cap of collagen, elastin fibrils, and smooth muscle cells over the lipid lesion. As discussed earlier, regional macrophages and activated smooth muscle cells over-express matrix-degrading enzymes (such as collagenases), and prothrombotic molecules[68] contribute to the progression of the atherosclerotic lesion. These atherosclerotic lesions also produce excess ROS that induce oxidative modification of LDLs and further endothelial dysfunction (Table 4).[26,28,69,70] These processes can contribute to plaque instability and thrombogenicity, resulting in the onset of acute coronary events.

Recognizing that atherosclerosis is a multi-factorial inflammatory process lends to the assumption that anti-inflammatory drugs and nutrients might mitigate the disease. It is interesting to note that many drugs used in the treatment of cardiovascular risk factors have anti-inflammatory properties by acting as antioxidants. The following are examples: angiotensin converting enzyme (ACE) inhibitors,[200] inhibitors of VCAM-1 (e.g., fibrates such as gemfibrozil),[201] inhibitors of inflammatory cytokine release (e.g., aspirin),[202] and lipid-lowering drugs (HMGCoA-reductase inhibitors).[203] All of these prevent lipoprotein oxidation and NO quenching. Similarly, nutrients with anti-inflammatory and antioxidant activity can contribute to the treatment of atherosclerosis.

Table 5. *Inflammation and Atherosclerosis – A Summary of Pathophysiology and Potential Nutrient Interventions*

Inflammation and its Actions	Processes that Modify Inflammatory Activity
Inflammation may determine plaque stability:[154,174] - Unstable plaques have increased leucocytic infiltrates - T cells and macrophages predominate rupture sites - Cytokines and metalloproteins influence both stability and degradation of the fibrous cap	Lipid lowering may reduce plaque inflammation by:[112,154] - Decreasing macrophage numbers - Decreasing the expression of collagenolytic enzymes (MMPs) - Increasing interstitial collagen - Decreasing the expression of E-selectin - Reducing calcium deposits
Inflammation increases the release of oxidant free radicals, which can lead to:[71,158] - Apoptosis - Leucocyte adhesion - Lipid oxidation and deposition - Vascular constriction - VSMC growth and matrix deposition - Thrombosis and platelet aggregation - Impaired NO metabolism - Cell phenotype change - Vascular leakage	Lipid lowering can be achieved by:[175-186] - Dietary modification - Supplementation with fish oil or omega-3 fatty acids (DHA/EPA)[150-153,182] - Increasing the intake of fiber[181,183] - Supplementing with niacin and vitamin C[185] - Statins - Tocotrienols[147-149]
Inflammation may be heightened by: - Improper balance between omega-3 and -6 fatty acids. Excess omega-6 fatty acids increases inflammatory response. - Exposure to trans fatty acids - Hyperglycemia, diabetes, smoking, chronic infection - Ischemic conditions - Advanced glycated end products - Hyperhomocysteine - Hormonal imbalance - LDL oxidation	Maintaining NO and oxidant balance by:[82,83,186-191,208] - Supplementing with arginine,[97,110,116,117] tetrahydrobiopterin,[187-190] vitamins B2, B3, and C and folic acid, which maintain NO synthesis[89,99,100,110,117] - Supplementing with antioxidant nutrients: vitamins C and E,[130-132,163,193-195] tocotrienols,[145,149] quercetin,[128,129,159,165,166] CoQ10,[167] lipoic acid, [120-123,170,171] superoxide dismutase,[131] and glutathione.[133-138] These antioxidants inhibit LDL oxidation, potentiate NO and prostacyclin synthesis, attenuate cell mediated LDL oxidation, inhibit agonist induced monocyte adhesion, decrease endothelial expression of adhesion molecules, reduce the proliferation of smooth muscle cells, and inhibit platelet aggregation.[196-199]

It can now be hypothesized that atherosclerosis may be an inflammatory disease that contributes to derangement of the vascular NO metabolic pathway and to increased oxidant stress. Most risk factors directly or indirectly influence this derangement and thus contribute to the expression of adverse cardiovascular symptoms (Figure 5). Fortunately, many nutrient factors can modify these risks and improve quality outcomes (Table 5).

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