Cardiovascular disease (CVD) is a universal problem in modern society. Atherosclerosis is the leading cause of CVD resulting in high rate of mortality in the population. Nutrition science has focused on the role of essential nutrients in preventing deficiencies, at the present time, the nutritional strategies are crucial to promote health and intervene with these global noncommunicable diseases. In many cases, diet is a major driving force, which is much easier to change and follow than other factors. It is important to establish that the first strategy to treat atherosclerosis is to modify lifestyle habits, focusing on the beneficial properties of specific nutrients. In the last decades, epidemiological, clinical and experimental studies have demonstrated that diet plays a central role in the prevention of atherosclerosis. In this review we will focus on the effect of specific foods, nutrients and bioactive compounds, including epidemiological facts, potential mechanisms of action and dietary recommendations to reduce the risk of atherosclerosis. In particular, we include information about fiber, plant sterols and stanols, niacin, taurine, olive oil, omega 3 fatty acids, antioxidants, minerals, methyl nutrients and soy. In addition, we also show that dysbiosis of the intestinal microbiota associated with a consumption of certain animal food sources can generate some metabolites that are involved in the development of atherosclerosis and its consequences on CVD. According to the epidemiological, clinical and experimental studies we suggest a recommendation for some dietary foods, nutrients and bioactive compounds to support the complementary clinical management of patients with atherosclerosis. © 2015 IMSS. Published by Elsevier Inc.
In the early steps of atherosclerosis, accumulation of foam cells evolves into fatty streaks. Complication of the lesion occurs when foam cells release growth factors and cytokines, which further stimulate VSMC migration from the media into the intima where they divide and produce ECM components such as collagen and contribute to the formation of a fibrous cap. If the pathological process persists and macrophages fail to remove accumulated cholesterol from the vessel, they become apoptotic, releasing cholesterol to the vessel wall and, more importantly, pro-thrombotic molecules and metalloproteinases (5). Progression and complication of atherosclerotic plaques are also characterized by a decreased number of VSMCs as well as the formation of immature and leaky new vessels, making atherosclerotic lesions more susceptible to rupture. Plaque disruption and the subsequent exposure of thrombogenic substrates initiate both platelet adhesion/activation and aggregation on the exposed vascular surface and the activation of the coagulation cascade, leading to thrombus formation and clinical manifestations of the atherosclerotic disease, acute myocardial infarction or sudden death (6,7).

A large part of the anti-atherogenic and anti-thrombotic properties of the vascular endothelium are mediated by its capacity to produce and release substances such as nitric oxide (NO), a platelet aggregation inhibitor with strong vasodilatory activity and an important anti-inflammatory function. NO blocks the expression of pro-inflammatory molecules such as necrosis factor kappa B (NF-kB) and adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) as well as leukocyte infiltration and adhesion. Tight and gap junctions are cell-cell connections with essential structures in regulating the endothelial permeability function. The formation of gap junctions is regulated by the presence and functionality of connexins, proteins whose expression is altered during the formation of atherosclerotic lesions. Gap junctions favor intercellular signaling processes and regulate NO-dependent vasodilation (1).

Atherogenic concentrations of LDL lead to a decrease in the bioavailability of endothelial NO. This decrease in NO availability is associated with a reduction in the concentration and/or activation of the endothelial NO synthase (eNOS), as a result of the presence of native LDL or modified LDL particles, as well as the degradation of NO through the formation of superoxide anions (O2−...). The imbalance between the redox state and NO is associated with protein nitrosylation. Deactivation of NO by O2 gives rise to highly cytotoxic peroxynitrite radicals. The increase of peroxynitrites derived from nitrotyrosines and the...

Figure 1. Effects of some nutrients and bioactive compounds on biological factors involved in the atherosclerotic process. ABCA1, ATP-binding cassette A1; AMPK, 5′ AMP-activated protein kinase; ACAT, acyl-coenzyme A:cholesterol acyltransferase; eNOS, endothelial nitric oxide synthase; FA, fatty acid; GSH, glutathione; HDL-C, high-density lipoprotein cholesterol; HMGCoAr, 3-hydroxy-3-methyl glutaryl-CoA reductase; ILs, interleukins; LDL-C, low density lipoprotein cholesterol; LOX1, oxidized LDL receptor 1; M1/M2, M1/M2 macrophages; NF-κB, necrosis factor κB; PKA, protein kinase A; SIRT1, sirtuin 1; SOD, superoxide dismutase; TNFα, tumor necrosis factor α; VSMCs, vascular smooth muscle cells.
production of O$_2^-$ are characteristics present in human atherosclerotic lesions (1,8,9).

Several studies have demonstrated that increased formation of reactive oxygen species (ROS) and/or altered oxygen utilization contributes to atherogenesis by superoxide production that mediates endothelial dysfunction and increases oxLDL levels. Small oxidized lipids that are components of ox-LDL, such as oxysterols, oxidized fatty acids and aldehydes, are potent inducers of ROS production (10). ROS in the vascular wall are generated by enzymes such as NADPH oxidase, xanthine oxidase and eNOS. Formation of intracellular ROS in the mitochondrial electron transport chain is controlled by antioxidant mechanisms. It has been shown that the increase of ROS generation by the mitochondria triggers cytochrome C release leading to caspase activation and apoptosis. The generation of large amounts of ROS can overwhelm the intracellular antioxidant defense, causing activation of neutrophils, protein modification, lipid peroxidation, and DNA damage, key factors for the initiation of atherosclerosis and the development of cardiovascular disease (CVD) (11).

Risk Factors for Atherosclerosis

The exact causes and risk factors of atherosclerosis are unknown; however, certain conditions, traits, or habits may raise the chance of developing atherosclerosis. Most risk factors including high total cholesterol and low-density lipoprotein cholesterol (LDL-C), low level of high-density lipoprotein (HDL) in the blood, hypertension, tobacco smoke, diabetes mellitus, obesity and sedentary lifestyle can be controlled and atherosclerosis can be delayed or prevented. Across cultures there are many different dietary lifestyles, some of which promote health and others that increase risk of atherosclerosis. There are some shared characteristics of healthy dietary pattern differences in cuisines worldwide that are associated with different macronutrient profiles. Although the emphasis on reducing saturated fat, trans-fat, and cholesterol to lower LDL-C, there is provocative evidence that other dietary constituents can reduce atherosclerosis in a manner independent of total cholesterol levels. As a result, there is keen interest in assessing the role of food-based bioactive compounds in reducing risk of atherosclerosis.
As mentioned above, diet plays a role in the development and treatment of CVD. The underlying molecular mechanisms by which dietary constituents contribute to the CVD pathology are thus an issue of paramount importance in the health area. The purpose of this review is to provide an overview of our present understanding of how foods and their bioactive components are involved in the prevention or treatment of atherosclerosis and discuss those whose excessive consumption favors the development of this disease.

The Mediterranean Diet

The outcome of the studies provoked the concept of cardioprotective properties of dietary habits within the Mediterranean regions in comparison with other regions of the Western world (12). This initial observation was further supported by many other epidemiological studies and several intervention trials. Among these, the recent clinical trial Primary Prevention of Cardiovascular Disease (PREDIMED) (13) and the Lyon Diet Heart Study (14) have robustly demonstrated the protective effects associated with the adherence of a Mediterranean-type diet in primary and secondary prevention of CVD. The Mediterranean diet (MeD) refers to a dietary profile commonly available in the early 1960s in the Mediterranean regions. It may be considered not one specific diet, but rather a collection of eating habits traditionally followed by people bordering the Mediterranean sea and consisting of a plant-centered diet with high intakes of vegetables and fruits (≥3 servings/day), whole-grain cereals (women 75 g/day, men 90 g/day), extra-virgin oil (≥4 tbsp/day), nuts (3–7 servings/week), moderate consumption of fish and poultry (≥3 servings/week), low intake of dairy products, red meat, and sweets, and moderate consumption of red wine for usual drinkers (≥7 glasses/week) (15). The Scientific Advisory Committee of the American Heart Association stated that the Mediterranean-style diet has impressive effects on CVD (6). The healthful properties of the MeD have been mainly attributed to the additive or synergistic interaction of its various constituents as a whole. Some MeD constituents have indeed been found to exert specific actions on the cardiovascular system, such as oleic acid (ω-9) and α-linolenic (ω-3) fatty acids, antioxidants and polyphenols, which have been shown to exert effects particularly on blood pressure, coagulation activity and endothelial functions. Later in this manuscript we will discuss each of them and their action in atherosclerosis.

Nutrients and Atherosclerosis

Fiber

Dietary fiber is a broad term for a variety of plant substances that are resistant to digestion in the human small intestine. They can be classified into two groups depending on their solubility in water. Common sources of soluble fiber include oats, pectin, psyllium, barley, flaxseed, and guar gum. Structural fibers such as cellulose, lignins, and wheat bran are insoluble.

Clinical Trial Evidence

Large population-based observational studies found that diets high in total dietary fiber are associated with a reduced CVD risk (16,17). An analysis of prospective cohort studies observed a 12% reduction in risk for coronary events and 19% for coronary deaths after the consumption of 10 g/day of dietary fiber (18). This could be explained by controlled studies, which have consistently demonstrated a reduction in LDL-C by ~5–7% with ingestion of soluble fiber (19). A meta-analysis shows that ingestion of soluble fiber (2–10 g/day) was associated with a significant 7% LDL-C reduction (20). The effect is independent of the type of soluble fiber including oat, psyllium, pectin, and guar gum. However, a dose-response relationship was noted with an absolute lowering of LDL-C by 1.12 mg/dL per gram of soluble fiber (21). It has been demonstrated that a combination of soluble fiber and soy protein significantly reduces LDL-C by ~20% after 2 months of consumption (22). Thus, clinical evidence indicates that reduction of LDL-C reduces the risk of atherosclerosis.

Proposed Mechanism of Action

Soluble fibers are thought to bind bile acids during the intraluminal formation of micelles in the intestine (23). This appears to be through physical entrapment rather than chemical binding. This leads to increased bile acid synthesis, reduction in hepatic cholesterol content, upregulation of LDL receptor (LDLr), and increased LDL clearance (20).

Other potential mechanisms include increasing intraluminal viscosity and slowing macronutrient absorption (24), entrapment of cholesterol in the small intestine, and increase in satiety leading to lower energy intake (25).

Dietary Recommendations

The adult treatment panel (ATP-III) recommends a daily intake of 5–10 g of soluble fiber as a therapeutic option to reduce LDL-C (19). A possible increase in flatulence may result from colonic fermentation. Symptoms are usually mild at the recommended doses of 5–10 g/day, and less fermented fibers may be helpful. In addition, gradually increasing the amount of dietary fiber and drinking adequate amount of fluids is recommended to limit symptoms.

Plant Sterols and Stanols

Plant sterols, or phytosterols, are naturally occurring sterols of plant origin with sitosterol being the most abundant. The
only difference between cholesterol and sitosterol consists of an additional ethyl group at position C-24 in sitosterol, which is responsible for its poor absorption (26). Vegetable oils are the main natural sources of plant sterols (27).

Clinical Trial Evidence and Clinical Implications

There is now evidence showing that plant sterols/stanols at dosages of 2–3 g/day decrease LDL-C by 6–15% (19). Studies are in agreement on the effectiveness of plant sterols in reducing LDL-C, independent of the food matrix used as vehicle. A meta-analysis showed that intake of 2.15 g/day of sterols reduced LDL-C by 8.8% and that greater intake did not significantly improve on this (28).

Proposed Mechanism of Action

Phytosterols compete with cholesterol for space within bile salt micelles in the intestinal lumen, thereby reducing cholesterol absorption (29). The presence of increased quantities of phytosterols in the gut lowers the micellar solubility of cholesterol, lowering the amount available for absorption (30). Another mechanism is their interaction with enterocyte ATP-binding cassette transport proteins to direct cholesterol back into the bowel (31). Phytosterols can be esterified to increase solubility and facilitate incorporation into fat-based food products.

Dietary Recommendation

The ATP-III recommends a daily intake of 2 g of plant sterol/stanol esters as a therapeutic option to reduce LDL-C (19). The vehicles used vary from fat-based food products such as spreads, milk, and yogurt to low-fat food products such as cereal, bread, and orange juice.

Niacin

Niacin, also known as vitamin B₃, is found in a variety of foods including liver, chicken, beef, fish, cereal, peanuts, and legumes and is also synthesized from tryptophan. In the Coronary Drug Project, niacin treatment was associated with a significant reduction in cardiovascular events and long-term mortality (32). Niacin increases high-density lipoprotein cholesterol (HDL-C) and reduces LDL-C levels, also favorably altering LDL particle size and number (33).

Clinical Trial Evidence and Clinical Implications

In clinical studies, niacin on average raised HDL-C levels 15–35%, lowered triglycerides (TG) 20–50%, lipoprotein (a) 24–38%, and LDL-C 5–25%. The National Cholesterol Education Program (NCEP) in 2002 recommended niacin alone to increase HDL levels when LDL levels are in the normal range (19). A recent meta-analysis revealed that niacin was associated with a significant reduction in non-fatal myocardial infarction without effect on cardiovascular risk when niacin is combined with a statin treatment (34).

Proposed Mechanism of Action

The mechanisms by which niacin impacts serum levels of HDL-C are complex and involve both synthesis and catabolism of this lipoprotein. Niacin increases the level and production rate of apolipoprotein A-I (ApoA-I) (35). In the human monocyte cell line, niacin increases expression of ATP-binding cassette subfamily A member 1, which facilitates transfer of cholesterol from macrophages to nascent HDL and thereby increases HDL-C (36). In human HepG2 cells and in cholesteryl ester transfer protein (CETP) transgenic mice, niacin reduces the catalytic rate of ApoA-I and inhibits CETP expression and activity (37,38). As CETP catalyzes 1:1 exchange of cholesterol ester (CE) from HDL in exchange for TG in very-low-density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and LDL, inhibition of CETP leads to increased CE contents of HDL and decreased catabolism of TG-rich HDL particles by hepatic lipase.

Taurine

Taurine is an amino acid-like compound present in substantial amounts in many mammalian tissues and is found in the cytosol of cells and in plasma. Taurine is not incorporated into protein structures and in humans it can be synthesized endogenously from methionine and cysteine primarily in the liver and delivered to target tissues by the circulation. Although humans can synthesize taurine, the majority is derived from food sources of animal origin, especially eggs, meat and seafood (39).

Clinical Trial Evidence and Clinical Implications

Worldwide epidemiological studies have indicated the beneficial effects of taurine intake on CVD prevention. The CARDiovascular Diseases and Alimentary Comparison (CARDIAC) study covering 61 populations worldwide found that taurine intake reduced CVD risk (40) and contributed to increased longevity in the Japanese population, which presented the lowest coronary heart disease mortality (41). Further studies revealed that 24-h urinary taurine, a marker of dietary taurine intake, was inversely associated with the age-adjusted mortality rates of stroke and coronary heart disease (42,43) and with lower body mass index, systolic and diastolic pressures, plasma total cholesterol levels and atherogenic index (44,45).

Proposed Mechanism of Action

Taurine uptake into the cells is mediated by a taurine transport system (46). Taurine is believed to have cardiovascular protective effects by the following five possible mechanisms: a) attenuation of vascular contractility by the
activation of the potassium channels K_{IR}, K_{ATP} and K_{Ca} (47) in several animal models like streptozotocin-induced diabetic mice (48) and rats (49); b) decrease of serum VLDL-C and LDL-C levels by improving LDLr binding capacity (50), reduction of apolipoprotein B-100 and VLDL secretion in HepG2 cells (51) and from the liver in rats (52,53) and mice (54) animal models, and activation of the bioconversion of cholesterol to bile acid via upregulation of cholesterol 7 alpha-hydroxylase expression (55); c) protection of vascular endothelial cells from apoptosis under hypertonic stress (56); d) reduction of ox-LDLs by decreasing the expression of oxidized LDL receptor-1 and soluble ICAM-1 (57); and e) reduction of the number of leukocytes and the migration of leukocytes to the vascular endothelium (58).

**Olive Oil**

Olive oil is the principal component of MeD. The protective role of olive oil against CVD can be seen in multiple studies. Concentration of the phenolic fraction in olive oil varies depending on the cultivar, climate, and degree of ripeness of the fruit. The average concentration is 500 mg/L in extra virgin olive oil. Of the various phenolic constituents of olive oil, hydroxytyrosol seems to be among the most important. However, olive oil phenols are a complex mixture of compounds (59).

**Clinical Trial Evidence and Clinical Implications**

In November 2004, the U.S. Food and Drug Administration approved a health claim for olive oil consumption (23 g/day) on the basis of its monounsaturated fatty acid (MUFA) content that had consistently demonstrated to benefit plasma lipid profile in clinical trials. More recently, the European Food Safety Authority released a health claim about the role of olive oil polyphenols (5 mg/day) in protecting LDL from oxidation in vivo. LDL oxidation is considered a key pathogenic event in atherosclerosis and subsequent coronary heart disease development (12). In fact, in subjects with a high cardiovascular risk, those with carotid intima media thickness (cIMT) showed a significant decrease when they consumed a diet enriched with olive oil for 1 year. Although most of the studies show a beneficial effect on CVD, one study with a small number of patients with angiographically documented coronary heart disease and normal plasma lipid levels supplemented with 6 g olive oil capsules for 28 months did not improve coronary atherosclerosis (60).

**Proposed Mechanism of Action**

Olive oil reduces LDL oxidizability in the postprandial state. In part, this reflects its fatty acid profile (high in MUFA) that is less susceptible to lipid peroxidation than polyunsaturated fatty acids.

**Omega 3 Fatty Acids**

Many epidemiological studies have accumulated evidence about the role of polyunsaturated fatty acids (PUFAs) such as ω-3 fatty acid (ω-3 FA), namely, α-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid, on the prevention and management of CVD (12). ω-3 FA is a class of essential PUFA with the double bond located in the third carbon from the methyl terminal position of the fatty acid (61).

**Clinical Trial Evidence and Clinical Implications**

cIMT is an early marker of atherosclerosis and is a predictor of cardiovascular risk, the higher the cIMT, the higher the risk of CVD. A number of cross-sectional studies have shown that ω-3 FA are inversely associated with cIMT. However, evidence from intervention trials does not support the epidemiological finding of the association between marine and plant-derived ω-3 FA with cIMT. In a cross-sectional study of middle-age Japanese males, the mean average cIMT was significantly higher in participants with a low fish consumption (<4 servings/week) compared to those with high fish intake (>4 servings/week). Results from this study suggest that high fish consumption (>4 servings/week) may be required to see an effect on cIMT (62).

**Proposed Mechanism of Action**

ω-3 FA modulates atherosclerosis by reducing the uptake and binding of LDL to the arterial wall. This is associated with a reduction of the arterial lipoprotein lipase levels and macrophage. ω-3 FA protects against inflammation in the arterial wall by reducing the production of proinflammatory cytokines in monocytes or macrophages and decreasing the recruitment of inflammatory leukocytes to the arterial wall (63). In addition, chylomicron remnants rich in saturated fatty acids compared with those rich in ω-3 FA were taken up more rapidly by cultured macrophages and resulted in greater arterial lipid accumulation (64). Furthermore, the antithrombotic properties of ω-3 FA are attributed to the incorporation of ω-3 PUFAs into the phospholipid membrane, altering the arachidonic acid metabolism and its subsequent reduction in thromboxane-A2 release (6).

**Antioxidant Compounds**

Free radicals are electrically charged molecules, which seek out and capture electrons from other substances to finally neutralize themselves. Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, resulting in a chain reaction. The term “antioxidant” refers to any molecule capable of stabilizing or deactivating free radicals before they attack cells. These include diet-derived antioxidants...
like ascorbic acid, vitamin E, carotenoids, and polyphenols, among others (11).

Vitamin E

Vitamin E comprises a group of eight abundant isomers (α-, β-, γ-, δ-tocopherol and tocotrienol) that differ according to their methylation patterns of the hydroxymethyl ring and saturation of the side chain. In vitro studies indicate that α-tocopherol is the most active isomer within the group of vitamin E (65).

Clinical Trial Evidence and Clinical Implications

Several meta-analyses suggest that vitamin E intake may prevent CVD and cardiovascular events (66). However, intervention studies provide evidence that supplementation with vitamin E does not reduce CVD incidence (67). However, it cannot yet be excluded that vitamin E intake is protective against CVD as primary prevention (68).

Proposed Mechanism of Action

α-Tocopherol reduces the stimulus-induced expression of adhesion molecules such as VCAM-1, ICAM-1 and E-selectin (69) and decreases the adhesion (70) and migration of leukocytes into the endothelium or into the arterial wall, respectively. α-Tocopherol makes it easier for endothelial cells to deal with oxidative stress such as oxygen peroxide (H₂O₂)-induced lipid peroxidation (71) due to higher catalase expression, increased H₂O₂ degradation activity, and higher intracellular glutathione (GSH) levels (72). Moreover, plaque stability is modulated by connective tissue growth factor, which stimulates the synthesis of extracellular matrix. This factor is induced by α-tocopherol in VSMCs (73), suggesting that it contributes to plaque stability by inducing fibrotic processes (68).

Vitamin C

Vitamin C (ascorbic acid) is a water-soluble vitamin involved in the formation of collagen and iron absorption. L-ascorbic acid is a dibasic acid with an enediol group built into a five-membered heterocyclic lactone ring that possesses antioxidant properties. Dietary sources of vitamin C include fruits and vegetables, particularly citrus fruits such as guavas and oranges.

Clinical Trial Evidence and Clinical Implications

Studies have shown that supplementation with > 500 mg vitamin C/day is associated with a lower risk of CVD, suggestive that higher doses of vitamin C are required to mediate protective effects. However, the secondary prevention trial Women’s Antioxidant Cardiovascular Study (WACS) did not find a benefit of 500 mg vitamin C/day on CVD (74). Nonetheless, a recent meta-analysis revealed that vitamin C supplementation improves endothelial function in atherosclerotic and heart failure patients, reducing CVD risk (75).

Proposed Mechanism of Action

Vitamin C has been shown to prevent apoptosis caused by cytokines in cultured endothelial cells. It also decreases the release of microparticles derived from endothelial cells and suppresses pro-apoptotic activity in congestive heart failure patients. Also, vitamin C stimulates all types of collagen synthesis by specific hydroxylase enzymes. Endothelial cell proliferation is, in part, associated with type IV collagen synthesis. On the other hand, vitamin C protects the vascular endothelium by enhancing eNOS. eNOS activity is inhibited by ROS that oxidize and deplete the cofactor tetrahydrobiopterin required for eNOS activity (76,77).

Lycopene

Lycopene is a carotenoid present in tomatoes and tomato products. Other minor food sources include grapefruit, watermelon, and papaya. Bioavailability is enhanced by cooking food sources of lycopene, particularly in the presence of oil or fats (59).

Several studies have reported that serum or tissue lycopene levels are inversely related to intimal wall thickness or lesions in the carotid artery and aorta, suggesting that lycopene may protect against the development of atherosclerosis. Because lycopene is an efficient antioxidant, it has been proposed that this property may be responsible for its beneficial effects (78).

Clinical Trial Evidence and Clinical Implications

In the European Community Multicenter Study on Antioxidant and Myocardial Infarction, adipose tissue lycopene concentration was independently protective against myocardial infarction (79). There is limited evidence that dietary supplementation of lycopene lowers LDL-C levels by ~14%, possibly because of an inhibition of cholesterol synthesis and increase in LDL degradation. In addition, lycopene reduces LDL oxidative susceptibility in vitro. There is also evidence that lycopene intake is associated with reduced intimal wall thickness and risk of myocardial infarction (80). So far, convincing evidence for the role of lycopene in protecting against atherosclerosis has not been provided, and there is a need for additional clinical studies to better establish the relationship between lycopene intake and atherosclerosis (78).
**Proposed Mechanism of Action**

The potential antiatherogenic role of lycopene has been ascribed mainly to its antioxidant capacity, which is related to the prevention of LDL oxidation. Another possible mechanism implicated in the reduction of intracellular cholesterol by lycopene is a decrease in cholesterol synthesis through inhibition of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase activity and expression, modulation of LDLr and inhibition of the enzyme acyl-coenzyme A:cholesterol acyltransferase activity (78).

**Resveratrol**

Resveratrol is a polyphenol found in the skin of grapes, berries and peanuts. It possesses antioxidant properties (81), increases the production of nitric oxide synthase (82) and improves mitochondrial function by activation of AMP-activated protein kinase (AMPK) and sirtuins (83).

**Clinical Trial Evidence and Clinical Implications**

A large amount of clinical trials have reported the role of resveratrol in several CVDs including hypertension, atherosclerosis and ischemic heart diseases (84). A meta-analysis of six randomized controlled trials investigated the effect of resveratrol on blood pressure and reported that high doses of resveratrol (>150 mg/day) significantly reduced systolic blood pressure, whereas lower doses had no effect (85).

Clinical trials that investigated the effect of resveratrol on plasma lipid profile in human subjects did not show clear effects. In patients with type 2 diabetes, a daily resveratrol dose of 250–1000 mg caused a significant reduction in LDL-C (86,87). In another study, daily ingestion of 350 mg resveratrol-enriched grape extract containing 8 mg resveratrol for 6 months caused a 20% reduction of ox-LDLs in patients treated with statins and with high risk of CVD (88). In contrast to these mentioned studies, resveratrol had no effect on lipid profile in several other studies (89–91).

The effect of resveratrol in patients with ischemic heart disease has been investigated in a few clinical trials. Oral administration of resveratrol, calcium fructoborate, and their combination was shown to improve several markers in patients with stable angina pectoris (92). In another study, patients supplemented with grape extract with 8 mg resveratrol for 6 months and 16 mg resveratrol for another 6 months increased levels of the anti-inflammatory adipokine, adiponectin (93) and a reduction in the thrombogenic plasminogen activator inhibitor type 1 (PAI-1) (94).

There is also a double-blind, placebo controlled, randomized clinical study examining the effects of resveratrol treatment in 40 patients with myocardial infarction. In this study, patients who consumed 10 mg/day resveratrol in conjunction with standard medication over 3 months had diastolic function and endothelial function significantly improved with a modest increase in systolic function. Platelet aggregation was inhibited and LDL-C levels were decreased (95).

**Proposed Mechanism of Action**

Resveratrol has been involved in the prevention of cardiovascular diseases including atherosclerosis by different mechanism of action.

1. Resveratrol induces sirtuins, which belong to class III histone desacetylases (96), have NAD+−dependent hydrolysis activity (97) and have a positive impact on cardiovascular physiology and physiopathology. These proteins are distributed in different subcellular compartments including the nucleus, cytoplasm and mitochondria where they regulate the expression and function of a variety of target genes and proteins (98). Particularly, activation of sirtuin 1 (SIRT1) by resveratrol induces plasticity of the vasculature by promoting the differentiation of VSMCs. This mechanism is dependent in the activation of AMPK (99).

2. Resveratrol also attenuates endothelial inflammation by preventing tumor necrosis factor alpha (TNFα)-induced inflammation and increasing autophagy through microtubule-associated protein 1 light chain 3 β2 expression and degradation of sequestosome 1 in human umbilical vein endothelial cells. This process is mediated by the induction of cyclic adenosine monophosphate (cAMP) abundance, protein kinase A (PKA), SIRT1 and AMPK activities (100).

3. Resveratrol counteracts with the proatherogenic oxysterol 7-oxo-cholesterol, the most abundant cholesterol autoxidation product in atherosclerosis lesion (101,102), which alters the macrophage subset M1/ macrophage subset M2 (M1/M2) balance favoring an inflammatory profile and contributing to the formation of the atherogenic plaque. In this mechanism, resveratrol controls the expression of differentiation and activation surface markers in M1 and M2 macrophages. It prevents the downregulation of cluster of differentiation (CD) 16, and the upregulation of the metalloproteinase matrix (MMP) 2 in response to 7-oxo-cholesterol in M1 macrophages, whereas it prevents the upregulation of CD14, MMP-2 and MMP-9, and the downregulation of endocytosis in M2 macrophages caused by 7-oxo-cholesterol (103).

**Minerals**

**Zinc**

Zinc is an important trace mineral, second only to iron in its concentration in the organism. It is found in cells...
Nutrition and Atherosclerosis

throughout the body and is needed for the proper function of the immune system, cell division, cell growth (104,105), and the breakdown of carbohydrates (106). Zinc is obtained in the diet mainly from animal tissue sources; optimal zinc status is an important consideration when evaluating the nutritional adequacy of vegetarian diets because of the absence of animal tissue sources of zinc and the increased intake of inhibitors of zinc absorption and bioavailability (107). More than 300 enzymes and >1000 transcription factors require zinc for their activities (108).

Clinical Trial Evidence and Clinical Implications

NF-κB, one of the major immune response transcription factors involved in molecular signaling, is regulated by zinc. A previous study in humans reported that normal healthy volunteers daily supplemented with 45 mg zinc as gluconate, have a significant decrease in the expression of TNF-α and interleukin (IL) 1β. TNF-α induces NF-κB DNA binding through upregulation of A-20, a zinc finger trans-activating factor that downregulates these cytokines. These effects were observed in isolated peripheral blood mononuclear cells compared to placebo treated subjects (109).

Proposed Mechanism of Action

Oxidative stress is an important contributor of atherosclerosis and CVD. Zinc functions as an antioxidant by different mechanisms: as an inhibitor of NADPH oxidase, which results in decreased generation of ROS; as a cofactor of the enzyme superoxide dismutase (SOD), which catalyzes the dismutation of oxygen to H2O2; by competing with Fe2+ and Cu2+ ions for binding to cell membranes and protein, displacing these redox active metals, which catalyze the production of OH from H2O2; by inducing the generation of metallothioneins, which are cysteine-rich proteins and are excellent scavengers of OH; by binding to sulfhydryl groups of bio-molecules protecting them from oxidation; and by increasing the activation of antioxidant proteins such as catalase, SOD and GSH (110).

Zinc is also a second messenger for immune cells. It is required for the development of monocytes/macrophages and regulates their functions such as phagocytosis and pro-inflammatory cytokine production (108). Further studies revealed that lipopolysaccharide stimulation of zinc-sufficient monocytes results in downregulation of inflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-8 (109,111,112). The mechanism is by the inhibition of the membrane phosphodiesterase, leading to elevated levels of the second messenger guanosine 3’,5’ cyclic monophosphate, which is followed by a subsequent suppression of NF-κB (111).

Zinc is involved in maintaining a healthy vascular condition. Vascular calcification is observed in atherosclerotic plaque and is a critical sign to transit to the severe stage of atherosclerosis. Atherosclerotic calcification of VSMCs occurs via the mechanism of cell death. Optimal zinc levels (15 μM) restore the decrease in VSMCs viability induced by calcifying conditions (113).

Potassium

Potassium is the most abundant intracellular cationic electrolyte necessary for normal cellular function; it also participates in protein synthesis and carbohydrate metabolism (114,115). Because potassium is readily excreted in the urine rather than stored in the body, the human body needs a constant intake of potassium in the correct amounts. Vegetables and fruits are typical examples of potassium-rich foods (116).

Clinical Trial Evidence and Clinical Implications

Several clinical trials have evidenced an inverse correlation between serum potassium levels or potassium intake and chronic diseases. In a multivariate analysis of 12,209 participants from the Atherosclerosis Risk in Communities (ARIC) study, an inverse correlation between serum potassium and risk of incident diabetes was found (117). In the fourth Korean National Health and Nutrition Examination Survey (KNHANES IV), an association was found between potassium intake and metabolic syndrome incidence. In this study, subjects in the highest quartile of potassium intake had 39% lower odds for metabolic syndrome compared to those in the lowest quartile, showing a significant inverse association between potassium intake and metabolic syndrome in adults (116). Dietary K+ supplementation is also demonstrated to lower arterial blood pressure in humans with essential hypertension (118–121). The Prospective Urban Rural Epidemiology (PURE) study found an inverse relationship between systolic blood pressure and urinary K+ excretion; with each gram increment of urinary K+ excretion, the associated systolic blood pressure dropped by 1.08 mmHg (122).

Endothelial dysfunction is an early event in atherosclerosis and can be measured before clinical manifestation of the disease. Blanch and co-workers (123) demonstrated that a high potassium meal, which contains a similar amount of potassium as 2.5 servings of bananas, can lessen endothelial dysfunction measured with the reduction in branchial artery by flow-mediated dilatation.

The Dietary Approach to Stop Hypertension (DASH) diet is rich in vegetables and fruits and part of its blood pressure-lowering effect can likely be attributed to its high K+ content (124). The intake of this diet is recommended to patients with cardiovascular disease because it significantly reduces blood pressure independently of the amount of dietary Na+ intake (120,125).

Proposed Mechanism of Action

High potassium diets have been shown to normalize blood pressure and to protect arteries from the development of 120,125.
atherosclerosis (126,127). The mechanism of blood pressure balance is driven in the nephron. Potassium (K\(^+\)) and sodium (Na\(^+\)) ions are freely filtered at the glomerulus and then reabsorbed to almost 90% along the proximal tubule and the thick ascending limb (128,129). Only 10% of the filtered Na\(^+\) and K\(^+\) load reach the distal tubule segments, which include the distal convoluted tubule (DCT), the connecting tubule (CNT), and the collecting duct (CD) with its cortical and medullary portion. These segments are decisive for the amount of Na\(^+\) and K\(^+\) that gets finally excreted via the urine (128–131). In the initial 2/3 of the DCT, the apical thiazide-sensitive NaCl cotransporter (NCC) is almost the sole apical Na\(^+\) transport pathway. In the last one-third of the DCT, NCC is co-expressed with the amiloride-sensitive epithelial sodium channel (ENaC) (131). The activity of ENaC is electrogenic and generates a transepithelial potential difference that is crucial to drive K\(^+\) secretion via apical K\(^+\) channels (132,133). K\(^+\) secretion is accomplished by the renal outer medullary K\(^+\) channel in the segment-specific principal cells of the DCT, CNT, and CD (134,135). Aldosterone is the main hormone mediating the effects of K\(^+\) intake on the kidney while also regulating Na\(^+\) balance and contributing to blood pressure control. A high dietary K\(^+\) intake increases plasma [K\(^+\)] and promotes aldosterone production (136). Aldosterone stimulates K\(^+\) secretion in the renal collecting system and interferes with the regulation of most of the molecular players involved in distal tubule K\(^+\) transport including ENaC (130), renal outer medullary potassium channel (137,138), and NCC (139,140).

In the atherogenic process, increase in potassium inhibits free radical formation from vascular endothelial cells and macrophages. It also contributes to inhibit the proliferation of VSMCs, platelet aggregation and arterial thrombosis (141).

**Magnesium**

Magnesium, the fourth most abundant cation in the human body and the second most abundant intracellularly, is involved in several essential physiological, biochemical, and intracellular processes regulating cardiovascular functions. It is widely distributed in plant and animal foods, in beverages and in dietary supplements. In general, foods containing dietary fiber provide magnesium. Green leafy vegetables such as spinach, legumes, nuts, seeds and whole grains are rich sources (142).

**Clinical Trial Evidence and Clinical Implications**

In epidemiological studies, low serum magnesium and dietary magnesium intake correlate with an increase risk in CVD in middle-aged adults from four U.S. communities in the ARIC Study (143) as well as in other prospective cohort studies (144). Results from the ARIC Study also show a correlation between low serum magnesium levels and incident kidney disease as a principal consequence of cardiovascular disease (145). In addition, experimental and clinical evidence suggest that both intra- and extracellular magnesium promotes vasodilation, reduces vascular resistance and improves blood flow system in cerebral, renal and coronary circulations (61,146). On the other hand, Kishimoto et al. (147) observed that supplementation with 500 mg of magnesium reduces serum and chylomicon TG in healthy human subjects.

**Proposed Mechanism of Action**

Magnesium could have a potential role in the prevention and treatment of atherosclerosis. It plays a critical role in modulating vascular smooth muscle tone, endothelial cell function, and myocardial excitability. Transcellular transport of magnesium in cardiac, renal and VSMCs involves efflux sodium-dependent systems and is implicated in the pathophysiology of hypertension (148).

Magnesium deficiency also potentiates free radical production and oxidative stress in endothelial cells through reduction in plasma antioxidants and increased lipid peroxidation (149,150). Low serum concentration of this ion impairs glucose homeostasis and action, elevates blood pressure, induces chronic inflammation and impairs vaso-motor tone (151). A previous study in Apo-E-deficient mice showed that animals consuming a low-fat diet and supplementation of drinking water with magnesium sulfate significantly reduce the median plaque area and cholesterol and triglyceride levels (152).

Hypomagnesaemia in rodents (153) and in humans with metabolic syndrome (154) provoke an elevation in TNF-\(\alpha\) serum concentration compared to healthy counterparts. It also exacerbates the stimulation of immune cells such as macrophages, T-helper cells and natural killer cells, which activate NF-\(\kappa\)B transcription factor and increase cytokine expression leading to inflammation (155).

**Methyl Nutrients**

Epigenetics can be defined as changes in gene expression that occur without changes in the DNA sequence. There are three major processes in epigenetics: DNA methylation, chromatin modifications (histone methylation, acetylation), and micro RNA (156). Epigenetics provide “marks” in the genome to activate or silence transcription, and these changes are heritable. Epigenomic marks are also responsive to environmental shifts like changes in nutritional status (157). Changes in DNA methylation patterns are involved in the prevention and treatment of CVD. Methylation of DNA involves the addition of methyl groups to the 5’ position of cytosine within CpG dinucleotides and is accomplished by DNA methyl-transferases (DNMTs) (158). Dietary factors required for methylation are often referred to as “methyl nutrients” and are necessary for the generation of S-adenosylmethionine (AdoMet), the
key donor for DNA, RNA, phospholipids and proteins (159). Methyl nutrients include vitamins such as folate, riboflavin, vitamin B₁₂, vitamin B₆, and choline and amino acids such as methionine, cysteine, glycine and serine.

**Folate**

Folate is a water-soluble B vitamin naturally present in a variety of foods; liver, yeast, mushrooms, and green leafy vegetables are the most significant. Most mammals are unable to produce folate except for the novo synthesis by intestinal microbiota. Therefore, the daily requirement must be obtained from dietary or supplementary sources (160). Tetrahydrofolate is the active form of folate and functions in the production of purines, pyrimidines and the remethylation of homocysteine to methionine.

**Riboflavin**

Riboflavin (vitamin B₂) is a water-soluble vitamin that is involved in a number of oxidative enzyme systems in electron transport (161). Flavin mononucleotide and flavin adenine dinucleotide are riboflavin coenzymes involved in diverse redox reactions in human metabolism and work as electron carriers (162) in carbohydrate, lipid, and other B vitamins and drug metabolism (163). Milk and milk products, whole egg, pork, and mandarin oranges are good sources of riboflavin.

**Vitamin B₁₂**

Vitamin B₁₂ (cobalamin) belongs to the “corrinoids” group, which comprises compounds that contain a corrin macrocycle (164). Cyanocobalamin, the most chemically stable and unnatural form of cobalamin, is included in most human dietary supplements and readily converted into the coenzyme forms of cobalamin. Methylcobalamin functions as a coenzyme for methionine synthase and is involved in methionine biosynthesis. 5′-Deoxyadenosylcobalamin functions as a coenzyme for methylmalonyl-CoA mutase and is involved in amino acid and odd-chain fatty acid metabolism in mammalian cells (165,166). Vitamin B₁₂ is well-known to be the sole vitamin that is absent from plant-derived food sources. Foods (meat, milk, eggs, fish, and shellfish) derived from animals are the major dietary sources of vitamin B₁₂ (164).

**Vitamin B₆**

Vitamin B₆ is water-soluble and consists of pyridoxine (PN), pyridoxamine, pyridoxal (PL), and their respective phosphate esters. Vitamin B₆ activates a number of coenzymes and is involved in numerous metabolic reactions. The most biologically active form of vitamin B₆ is pyridoxal 5′-phosphate, which is a cofactor for transaminases, decarboxylases, racemases, and other enzymes used in the metabolic transformations of amino acids and nitrogen-containing compounds (167). Vitamin B₆ is widely distributed in foods from plant and animal origin. It is found in meats and eggs and in plant foods such as beans, cereals, and brown rice. PL predominates in animal products and PN in plant foods. Plant sources are generally less bioavailable than animal sources because plants contain dietary fiber causing incomplete digestion and less bioavailable glycosylated forms of PN (168).

**Choline**

Choline is a water-soluble vitamin group in the B-complex vitamins. It is found in a wide variety of foods; the main sources are soy, beef liver, milk, chicken, broccoli, cauliflower, and egg yolks (169). It is needed for the synthesis of the neurotransmitter acetylcholine and is required to make the phospholipids phosphatidylcholine, lysophosphatidylcholine, choline plasmenogen, and sphingomyelin, essential components for all membranes (170). Choline is the major dietary source of methyl groups via the synthesis of AdoMet. Upon entry into the cell, choline is immediately phosphorylated to phosphocholine or oxidized to betaine in some cell types such as hepatocytes (171). Betaine is used for methionine synthesis through homocysteine reduction (172).

**Clinical Trial Evidence and Clinical Implications**

Several clinical trials consistently show a reduction in homocysteine levels followed by supplementation with folate and vitamins B₆ and B₁₂ (173–175). Plasma total homocysteine is associated with an increased risk of coronary artery disease (176). Folic acid is the fully oxidized monoglutamyl form of folate and is frequently used as the nutritional supplement of folate due to its stability and bioavailability (160). Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of eNOS, the enzyme catalyzing the formation of NO, and is a mediator of atherogenic effects of homocysteine. Supplementation with folic acid and vitamin B₁₂ significantly reduced the plasma levels of ADMA and homocysteine increasing NO synthesis in patients with acute ischemic stroke, suggesting a new pathway of prevention of atherogenic effects involving NO-related mechanisms (177). Interestingly, Mexican women consuming an urban diet show higher plasma homocysteine levels compared to those ingesting a rural diet (178).

Supplementation with folic acid and vitamins B₆ and B₁₂ significantly reduces cIMT and its progression in patients with cerebral ischemia, chronic kidney disease, and cardiovascular risk (177). Garaiova and coworkers (179) found that an emulsified combination of sterols, fish oil and B vitamins including...
vitamins B₆, B₁₂ and folate has beneficial effects on cardiovascular risk factors by reducing total cholesterol, LDL-C, VLDL-C, IDL-1 and IDL-2 subfractions, and homocysteine levels in hypercholesterolemic children and adolescents during the short-term.

**Proposed Mechanism of Action**

Methyl nutrients are metabolically required for the generation of AdoMet by three pathways: trans-sulfuration pathway, folate cycle and methionine cycle. B vitamins lower homocysteine levels by promoting its metabolism. Homocysteine can be removed from the circulation by catabolism to cysteine and this reaction uses vitamin B₆. It could also be remethylated to methionine and this process requires vitamin B₁₂ and riboflavin as cofactor. Betaine (endogenously synthesized from choline) or folate, can donate the methyl group to make the reaction (180). Methionine can be converted to AdoMet by methionine adenosyltransferase and the reversible reaction produces S-adenosyl-homocysteine (AdoHcy) following methyl donation by liberation of adenosine and formation of homocysteine catalized by AdoHcy hydrolase (156).

Most studies investigating nutrient imbalance and DNA methylation have focused on folate status and hyperhomocysteinemia. Homocysteine is a sulfur-containing amino acid associated with cardiovascular risk and formed during the metabolism of methionine. Homocysteine results in the production of hydrogen peroxide and oxygen-based free radicals (superoxide and hydroxyl radical), inducing endothelial cell injury and dysfunction via oxidative stress (3–6,23). Early atherosclerotic lesions in hyperhomocysteinemic patients mainly depend on damage or impaired regeneration of the endothelial cells induced by DNA hypomethylation. Hyperhomocysteinemia also reduces AdoMet/Ado Hcy ratio (181–183). In vitro studies revealed that an increase in AdoHcy concentration inhibits DNMTs in the liver (184) and in cultured human fibroblast (185) indicating a reduction of DNA methylation capacity. In endothelial and VSMCs DNA hypomethylation of the monocyte chemotactic protein 1 promoter through NF-kB/DNMT1 can influence monocyte recruitment and inflammation (186). In addition, DNA hypomethylation of Kruppel-like factor 2, a transcription factor involved in endothelial vascular homeostasis, can cause downregulation of its target genes such as thrombomodulin, eNOS and PAI-1 promoting platelet aggregation (187).

In an apolipoprotein E (ApoE)-deficient mice atherosclerotic model, folic acid supplementation has a positive impact on homocysteine levels and atherosclerotic lipid levels as a treatment to prevent plaque area progression (188). On the contrary, moderate but prolonged folate deficiency significantly increases plaque formation in the aorta of ApoE null mice fed a high-fat diet, indicating a positive role for folate in modulating vascular disease in vivo. However, this effect is not associated with changes in intermediates in the methionine cycle or with global DNA methylation status in the liver or vasculature of these animals (189).

Moreover, a recent study shows that folic acid exerts anti-angiogenic activity. In this mechanism, folic acid binds to its receptor (folic acid receptor) and subsequently activates the extracellular-signal-regulated kinases/NF-κB/-signaling pathway which, in turn, upregulates the expression of p21 and p27 and finally results in cell cycle arrest at the G0/G1 phase inhibiting vascular endothelial cell proliferation (160).

**Soy**

The soybean is a legume native to East Asia that has numerous uses. The plant is classified as an oilseed by the U.N. Food and Agricultural Organization and contains a considerable amount of polyunsaturated fatty acids. Soy protein is used as a substitute for animal products because it has a complete protein profile providing all the indispensable amino acids in the amounts needed for human health. Soy products also contain significant amounts of the isoflavones genistein and daidzein either in an unconjugated aglycone form or in different glycoside conjugates (190), which provide several beneficial effects according to clinical and experimental animal studies.

**Clinical Trial Evidence and Clinical Implications**

The atheroprotective effects of soy-based diets have been attributed to the associated reduction in cholesterol levels and reduction in oxidative stress in human studies. There is epidemiological evidence that intake of soy products is associated with a lower incidence of CVD in Asian populations (190). The Framingham Offspring Study highlighted a favorable cardiovascular risk profile in postmenopausal women receiving isoflavone supplements (191); however, the American Heart Association attributes the benefits of isoflavones to the high content of polyunsaturated fats, fiber, vitamins, minerals, and low content of saturated fat in soy products (192). In hypercholesterolemic subjects, a low saturated fat diet (LSFD) for 1 month followed by a LSFD and supplementation with 25 g of soy protein and 15 g of soluble fiber daily for 2 months reduce serum LDL-C by 25.2% (193), serum total cholesterol 20.6%, and serum TG by 40.4% (22). In addition, the consumption of a dietary pattern with soy protein, nopal, chia seed and oat, decreases serum TG, C-reactive protein, and area under the curve for insulin and glucose intolerance after a glucose tolerance test (194).

**Proposed Mechanism of Action**

Consumption of soy protein significantly reduced serum cholesterol, especially LDL-C. Studies in rats fed soy diets...
show lower hepatic sterol regulatory element binding protein-1 (SREBP-1) expression and higher SREBP-2 expression than those fed casein diets, leading to less hepatic lipid deposition. In addition, soy-fed rats have less LDL-C levels (195,196). Soy protein intake reduces ceramides, cholesterol and triglyceride concentrations in hearts of rats and ob/ob mice due to an increase in the oxidation of fatty acids regulated by the peroxisome proliferator activated receptor-γ transcription factor accompanied by a reduction in the lipogenic transcription factor SREBP-1. In addition, serine palmitoyl transferase 1 and TNF-α mRNAs concentrations mRNA are also reduce in soy protein fed animals compared to those fed a casein diet (197).

Dietary soy inhibits atherosclerotic lesion development by mechanisms other than lowering serum cholesterol. Studies in ApoE knock-out mice showed that atherosclerotic lesions are reduced when fed a soy-containing diet despite uncharged serum lipid levels (198). Isoflavone supplementation provides protection against oxidative stress in the cardiovascular system and other organs enhancing the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) activity, a transcription factor that protects the aging vasculature by upregulating the transcription of genes of antioxidant enzymes preventing atherogenesis (198–200). However, Nrf2 also mediates induction of CD36 and LDL scavenger receptor (201) and paradoxically makes atherosclerotic lesion progression significantly worse due to increased uptake and accumulation of lipids within macrophages via Nrf2-mediated CD36 regulation and the formation of foam cells (202), despite the fact that inflammation and oxidative stress levels are diminished. However, this effect requires further investigation to ascertain if upregulation of Nrf2 in the vasculature by isoflavones has similar consequences.

Compounds in soy are also known to regulate vascular tone. Studies with human fetal endothelial cells have shown that genistein, daidzein and equol (1–100 nM) activate eNOS (203). However, higher concentrations of genistein (10–50 IM) inhibit tyrosine kinase activity (204), thus vascular actions of higher concentrations of isoflavones result from non-selective kinase inhibition (205–207). Studies in bovine aortic endothelial cells have shown that genistein elicits eNOS phosphorylation after activation of PKA (208) and the daidzein metabolite equol also phosphorylates eNOS Ser1177 by the activation of ERK1/2 and PI3-kinase/Akt pathways stimulating endothelial NO production. In addition, high genistein concentrations (50 IM) potentiate the activity of a transient receptor potential channel 5 cation modulating release of vasodilators, vascular permeability and response of the endothelium to inflammatory mediators in bovine aortic endothelial cells (209).

**Gut Microbiota and CVD**

The intestinal microbiota influences the physiology and metabolism in the body and the response to dietary changes. Recent evidence implicates the gut microbiota in the susceptibility to develop nonalcoholic fatty liver, insulin resistance, obesity and CVD (210). In addition, susceptibility also depends on the intersection between host genotype and diet to influence host metabolism. Gut microbiota produces several metabolites in response to the host diet. Metabonomic approaches identified small molecules that act as potential triggers for CVD. Trimethylamine-N-oxide (TMAO), which is a metabolite of dietary choline and L-carnitine, is a candidate of susceptibility for CVD (211). L-carnitine, a particularly abundant nutrient in red meat, contains a trimethylamine structure very similar to choline (212). The quaternary amine structure of L-carnitine is converted to trimethylamine (TMA) by gut microbes, which is subsequently converted by the host flavin monoxygenase (FMO) enzyme family to TMAO in the liver (213).

**Clinical Trial Evidence and Clinical Implications**

In a previous prospective clinical study, ingestion of two hard-boiled eggs, each containing about 250 mg choline, and 250 mg of deuterium-labeled phosphatidylcholine, TMAO and deuterium-labeled TMAO were detected in the plasma after a dietary phosphatidylcholine challenge (214). In another clinical study, fasting plasma TMAO levels were associated with a major incident of adverse cardiovascular events (MACE, defined as death, myocardial infarction or stroke) over a 3-year follow-up in 4007 patients who underwent elective coronary angiography (211). Plasma L-carnitine levels predict an increased risk of CVD and MACE in a large clinical cohort (n = 2,595), but only in subjects whose TMAO levels are elevated. Moreover, comparison of omnivores with frequent dietary L-carnitine consumption vs. vegans or vegetarians reveals a striking difference in the capacity to convert dietary L-carnitine into TMA and TMAO, with vegans/vegetarians showing virtually no TMA/TMAO formation (213).

**Proposed Mechanism of Action**

TMAO can impact distinct steps in cholesterol and sterol metabolism and macrophage foam cell formation. Dietary choline or TMAO supplementation increases the expression of the scavenger receptors (CD36 and scavenger receptor type 1) on macrophages and reduces reverse cholesterol transport, promoting foam cell formation (211,215). Direct dietary exposure to TMAO or its precursors, choline or L-carnitine, elicit a significant reduction in reverse cholesterol transport in vivo in mouse models as well as alterations in cholesterol and sterol metabolic pathways in multiple compartments including the artery wall, the liver, and the intestine (211,215,216). TMAO levels explain a significant portion of atherosclerosis development across multiple different inbred strains of mice. TMAO levels are influenced by the hepatic farnesoid X receptor, a bile acid-activated nuclear receptor (214), because it regulates the
expression of the enzymes required for its biotransformation from TMA.

Cellular L-carnitine levels are significantly higher than plasma L-carnitine levels; thus, L-carnitine is actively transported into cells in a slow sodium-dependent manner (217,218). However, the transport of L-carnitine into microvascular cells remains to be determined. Choline and L-carnitine are converted to TMAO in the gut microbiota. Choline and betaine are released by hydrolysis of phosphatidylcholine to TMA. Dietary L-carnitine is metabolized by the gut microbiota into γ-butyrobetaine that is further converted into TMA. TMA is then oxidized by the enzyme FMO in the liver to TMAO and released into the circulation. TMAO can also be degraded by some bacteria into dimethylamine, methylamine, and finally ammonia, and all of these metabolites can be absorbed and further metabolized endogenously by the liver (219).

Conclusions

Atherosclerosis is one of the most important risk factors for the development of CVD. Thus, several pharmacological strategies have been used to decrease or prevent the development of atherosclerosis. Interestingly, in recent years several studies have emphasized the role of several dietary nutrients and bioactive compounds in the mechanisms of the processes around the plaque formation. Nutrients such as saturated fatty acid and trans-fatty acids, among others, are associated with an increase in the development of atherosclerosis. On the other hand, nutrients such as soy, omega-3 fatty acids, several vitamins, and polyphenols reduce or attenuate the appearance of atherosclerotic lesions. The activity of these compounds is associated with a reduction in the inflammatory response, in the antioxidant capacity to prevent oxidation of LDL particles, leukocyte migration, adhesion molecules, in the viability of VSMCs and reduction in blood pressure among others. Furthermore, it has recently been shown that the microbiota may play an important role in risk of CVD through the formation of specific metabolites that may regulate in a direct or indirect way the formation of atherosclerotic plaques. Therefore, there is a need in the field of nutrition to establish appropriate recommendations on the intake of these nutrients and bioactive compounds in order to reduce the risk of CVD. It is important to conduct new clinical randomized trials using the combination of specific foods, nutrients or bioactive compounds that synergistically improve the physiological conditions of capillaries and blood vessels in order to reduce the risk of CVD.

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