

Antioxidants, Diabetes, and Endothelial Dysfunction

a report by

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A substantial amount of evidence has demonstrated that diabetes is highly associated with oxidative stress and endothelial dysfunction.¹⁻³ It is also well recognized that endothelial dysfunction, which is present even in people at risk of developing diabetes, is strongly connected with oxidative stress and considered as a preliminary risk factor for the development of atherosclerosis and cardiovascular disease.^{4,5}

Given the central pathogenic role of the dysfunctional endothelium in the atherosclerosis of large- and medium-sized arteries,^{6,7} it is increasingly clear that endothelial cells are the ultimate target for pro-atherogenic mediators in oxidative stress, insulin resistance, and diabetes.⁷⁻⁹ Therefore, there is growing research in the role of antioxidants on endothelial function as a new therapeutic approach for reducing the risk of cardiovascular disease in patients with diabetes.

This report will describe the association between endothelial dysfunction and oxidative stress in diabetic patients. It will also provide the latest data about the role of antioxidants as a new therapeutic intervention for the reduction of cardiovascular risk in diabetic patients.

Diabetes and Oxidative Stress

Diabetes is currently recognized as an oxidative stress disorder.¹⁰ Oxidative stress *per se* is characterized by high accumulation of reactive oxygen species (ROS) that cannot be coerced by the endogenous circulating neutralizing agents and antioxidants. Hyperglycemia can induce oxidative stress through four mechanisms:

- increased production of advanced glycation end-products (AGEs);
- increased flux through the polyol/aldose pathway;
- activation of protein kinase C (PKC); and
- increased flux through the hexosamine pathway (see *Figure 1*).

Excessive superoxide production by the mitochondrial transport chain¹¹ during hyperglycemia may be the initiating factor of all the above-mentioned processes. The formation of superoxide and the subsequent increase in oxidative stress decreases nitric oxide (NO) bioavailability, resulting in endothelial dysfunction and ultimately leading to atherosclerosis and cardiovascular disease.¹²

Hyperglycemia promotes oxidative stress by contributing to the production of AGEs, which are non-enzymatically glycosylated proteins or lipids susceptible to oxidation after exposure to aldose sugars.¹³ AGEs can produce ROS and trigger mechanisms that generate the production of intracellular oxidants. In addition, AGEs have been found to alter

extracellular matrix protein function, cause vascular leak, decrease the bioavailability of endothelium-derived NO, and promote inflammation.¹⁴

Hyperglycemia can also promote oxidative stress by increasing polyol pathway flux.¹⁵ Although aldose reductase usually presents low affinity to glucose, in a high-glucose-concentration environment the increased intracellular glucose results in an increased activity of aldose reductase and a consequent increase of the glucose reduction to sorbitol. This procedure, which consumes nicotinamide adenine dinucleotide phosphate-oxidase (NADPH), decreases the reduced glutathione, subsequently increasing oxidative stress.¹¹

Furthermore, hyperglycemia and elevated free fatty acids increase PKC activity,¹⁵ which promotes oxidative stress through activation of mitochondrial NADPH oxidase. Increased PKC activity promotes vascular occlusion, and vascular inflammation by decreasing NO production and increasing endothelin-1 (ET-1) production and vascular permeability.^{10,16} In addition, hyperglycemia shunts excess glucose through the hexosamine pathway.¹⁷ Excessive intracellular glucose results in conversion of fructose 6-phosphate to glucosamine 6-phosphate, promoting a series of reactions that increase oxidative stress by NADPH depletion, transforming growth factor-beta (TGF-β), and plasminogen activator inhibitor-1 (PAI-1) gene expression increase and endothelium nitric oxide synthase (eNOS) activity inhibition.¹⁸



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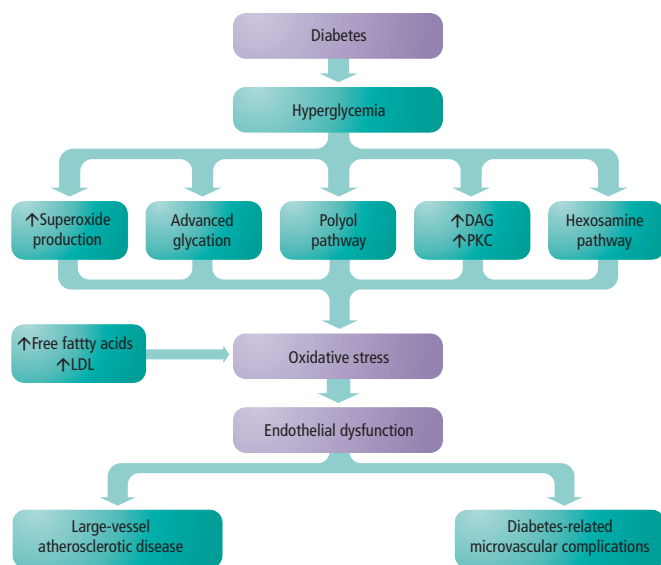


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Figure 1: The Pathogenesis of Oxidative Stress in Diabetes



Oxidative Stress and Endothelial Dysfunction

Endothelial dysfunction, first described in the early 1980s by Furchgott and Zawadzki, reflects an imbalance between the release of vasodilator and vasoconstrictor endothelium-derived factors. This imbalance is thought to principally involve the reduced bioavailability of NO resulting from its rapid inactivation by endothelial production of ROS.^{19,20} Reductions in vascular NO signaling mediated by ROS may be accompanied in diabetes by a reduced synthesis of prostacyclin,^{21,22} coupled with an undiminished or even enhanced formation of vasoconstrictor agents, which is also a factor in restricted vasodilation.^{23,24} With increased oxidative stress, tetrahydrobiopterin (BH4), a co-factor that regulates NO production tightly, is oxidized resulting in the uncoupling of eNOS and reduced NO production.²⁵ Elevated levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of eNOS through competition with L-arginine, may further reduce NO production.²⁶ This perpetuates a cycle of vascular oxidative stress through the transfer of electrons to molecular oxygen, forming oxidant species such as superoxide and peroxynitrite, which further consume NO and increase oxidative stress, leading to endothelial dysfunction.^{25,27}

Endothelial Dysfunction in Diabetes

Endothelial dysfunction has been demonstrated in type 2 diabetes in both the resistance and conduit vessels of the peripheral circulation,²⁸⁻³² as well as in the coronary circulation.^{33,34} The soluble adhesion molecules E-selectin, vascular cellular adhesion molecule (VCAM)-1, and intercellular adhesion molecule (ICAM)-1 are elevated in subjects with type 2 diabetes.³⁵⁻³⁸ Similarly, increased levels of von Willebrand factor (vWF), a measure of endothelial cell damage and activation, are found in diabetes.^{35,37,38} Microalbuminuria is an independent predictor of endothelial dysfunction and may indicate widespread vascular dysfunction in diabetes.^{35,39}

Although the pathogenetic mechanisms underlying the development of endothelial dysfunction in diabetes are not yet fully identified, according to the above-mentioned data they mainly involve increased oxidative stress and the subsequent restriction in NO bioavailability.

Antioxidants

Since oxidative stress is the main cause of endothelial dysfunction in patients with diabetes, strategies targeting reactive oxygen species reduction seemed to be a new potential treatment for diabetic endothelial dysfunction and, consequently, a treatment for diabetic vascular complications. In this direction, many studies have examined the possible beneficial effect of antioxidant substances in endothelial function in both animal and human subjects with diabetes.

Animal Studies

The majority of studies in diabetic animal models had favourable outcomes, presenting a significant reduction of the oxidative stress after the administration of antioxidative substances. Specifically, vitamin E averted endothelial dysfunction in cholesterol-fed rabbits and streptozotocin-diabetic rats.^{40,41} However, some studies reported that antioxidant vitamin supplementation may lead to endothelium dysfunction in both diabetic and normal animals.^{42,43} Proposed mechanisms for this observation include pro-oxidant effects of vitamin E on vitamin C in the presence of NO and/or *de novo* synthesis of vasoconstrictive prostanoids.⁴⁴ Differing levels of vitamin E and C prior to repletion may also play a role. Tetrahydrobiopterin (BH4) is an essential co-factor of the NOS.⁴⁵ BH4 is best known for stabilizing NOS dimmers, preventing the uncoupling of NOS and the subsequent superoxide formation. As uncoupled NOS is a large source of ROS, BH4 deficiency is an important mechanism in promoting oxidative stress.⁴⁶ Furthermore, oxidative stress decreases the bioavailability of BH4, furthering superoxide formation and decreasing NO production. Studies in diabetic rats have shown that BH4 supplementation ameliorated endothelium dysfunction.⁴⁷ Benfotiamine, a lipid-soluble thiamine derivative, has also been shown to have significant antioxidant effects. Thiamine and benfotiamine activate the enzyme transketolase, which diverts glucose metabolites away from the mediators of oxidative stress, namely the polyol, hexosamine, diacylglycerol (DAG), and AGE pathways. In animal models, benfotiamine inhibited diabetic retinopathy and reversed the toxic effects of glucose on endothelial progenitor cell (EPC) differentiation.^{48,49} Lipid-lowering agents such as hydroxymethylglutaryl-co-enzyme A (HMG CoA) reductase inhibitors (statins) have also demonstrated potential antioxidant effects. However, as the main mode of action of these medications is not the reduction of oxidative stress, they will not be reviewed in this article.

Human Studies

Vitamin C

A substantial amount of human research has focused on the vitamin antioxidants such as vitamins B, C, and E. Due to their known superoxide scavenger properties, vitamins C and E have been widely investigated and tested either alone or in combination. Initial studies involving acute increase of the vitamin C plasma levels presented a significant improvement of endothelial function in multiple disease models of oxidative stress. More specifically, Beckman et al. reported that hyperglycemia-induced endothelial dysfunction in non-diabetic subjects was reversed by vitamin C infusion.⁵⁰ Intra-arterial infusion of vitamin C has been shown to improve endothelial function in both types I and II diabetic patients.^{51,52} Other studies have shown similar results in subjects with essential hypertension, reporting that vitamin C infusion resulted in an immediate endothelial function improvement, whereas other antioxidants such as n-acetylcysteine did not have the same effect.⁵³ Although acute studies have shown significant

improvement in endothelial function with vitamin C, long-term antioxidant therapy has not been as successful. In a recent study, combined therapy with vitamins C and E in types I and II diabetic patients showed an improvement in endothelial function only in type I diabetic patients.⁵⁴ In another study, high oral doses of vitamin C did not improve endothelial function in type II diabetic subjects.⁵⁵ Interestingly, in this study, although the diabetic patients with low vitamin C levels were able to replenish their vitamin C levels with the high-dose oral supplementation, they did not achieve as high levels as healthy non-diabetic subjects. As high concentrations of vitamin C are needed to compete for superoxide, the failure to achieve these levels was proposed as the main reason for the vitamin C failure in this group of patients.⁵⁶

In summary, according to the current data there is no compelling evidence to support the use of vitamin C for preventing cardiovascular disease in diabetes.

Vitamin E

High doses of vitamin E supplementation have not shown any clear beneficial effects in surrogate measurements of cardiovascular disease in diabetics. A study from our unit, which included both types I and II diabetic patients treated with a high dose of vitamin E (1,800IU daily) for 12 months, found no improvement in endothelial-dependent or -independent vasodilation in both skin microcirculation and brachial artery macrocirculation tests.⁵⁷ In addition, left ventricular function was not affected by vitamin E supplementation. Endothelin (a potent vasoconstrictor) was increased in the treatment group after six months (but normalized by 12 months), while endothelial-independent vasodilation and the systolic blood pressure worsened slightly by the end of the 12-month treatment period. Of interest, C-reactive protein (CRP), a marker of inflammation, was decreased in the vitamin-E-treated group. Thus, although vitamin E may present a beneficial anti-inflammatory effect, it does not seem to have a positive effect on cardiovascular function. A number of large clinical trials that have employed clinical end-points have also been conducted. One of the initial studies, the Cambridge Heart Antioxidant Study (CHAOS), which employed vitamin E 400–800IU reported a significant risk reduction from non-fatal myocardial infarction after an 18-month follow-up period. This reduction was accompanied by a non-significant excess of cardiovascular deaths in the same group. Subsequent large-scale trials failed to yield similar beneficial outcomes.⁵⁸ A large Italian study, the GISSI-Prevenzione trial, employed vitamin E 300mg per day and n-3 polyunsaturated fatty acids (PUFAs) or placebo for a median of 3.5 years.⁵⁹ Vitamin E had no effect in preventing cardiovascular outcomes, in contrast to n-3 PUFA, which had a clinically important and statistically significant benefit. The largest North American trial to date has been the Heart Outcomes Prevention Evaluation study (HOPE), which was published in 2000.⁶⁰ It enrolled over 9,500 subjects aged 55 years and above, 3,654 of whom had diabetes, and employed 400IU vitamin E daily for a mean follow-up of 4.5 years. Vitamin E had no effect on cardiovascular outcomes in all subgroups, including those with diabetes. The HOPE trial was subsequently extended to the HOPE—The Ongoing Outcomes (HOPE-TOO) trial, which included a large number of the participants of the original study (7,030 patients, of whom 2,680 had diabetes).⁶¹ Subjects were followed for an average of seven years in this study and were randomized to placebo or 400IU vitamin E daily. There was no difference in cardiovascular outcomes (including myocardial infarction,

stroke, and death from cardiovascular causes) between the treatment and placebo groups. Surprisingly, subjects treated with vitamin E had higher rates of heart failure and heart-failure-related hospital admissions. This finding was consistent among all subgroups, including diabetes, and was persistent through both HOPE and HOPE-TOO. Although the mechanism related to the vitamin-E-associated excess rate of heart failure was not clear, the HOPE-TOO investigators hypothesized that one possibility could be that vitamin E may be pro-oxidative in certain circumstances, subsequently depressing myocardial function. Although initial meta-analyses did not show any effect of vitamin E on survival, one common shortcoming in these studies was that the effect of dose was not analyzed.^{62,63} The dose was taken into account in a recent meta-analysis that included 19 clinical trials and examined the relationship between vitamin E supplementation and total mortality. The results showed that in nine of 11 trials testing high-dose vitamin E (≥ 400 IU/d), the all-cause mortality risk increased, prompting the conclusion that high doses of vitamin E (≥ 400 IU/d) should be avoided.⁶⁴ In conclusion, as the data indicate, there is currently no compelling evidence to support the use of vitamin E for preventing cardiovascular disease in diabetes. In addition, high doses of vitamin E may be associated with serious side effects. Thus, it is reasonable to suggest that such high doses should be avoided.

Tetrahydrobiopterin (BH4)

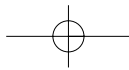
The bioavailability of BH4 decreases during oxidative stress conditions (such as hyperglycemia), which in turn reduces endothelial NO production and subsequently causes endothelial dysfunction. Repletion of BH4 is believed to improve the production of NO because it is an essential co-factor of the NO synthetase. In healthy adults, oral-glucose-induced endothelial dysfunction was reversed acutely with tetrahydrobiopterin (BH4).⁶⁵ Furthermore, in subjects with type II diabetes, infusion of BH4 was shown to improve endothelial function acutely.⁶⁶ Currently, there are no clinical trials that have examined the effect of BH4 on cardiovascular risk in diabetes, and further research in this field is required before any recommendations can be made.

Benfotiamine

Although large-scale clinical trials using benfotiamine are lacking, small studies have shown promising results. Thus, a recent small clinical trial examined the effect of three-day therapy with benfotiamine 1,050mg daily on macro- and microvascular endothelial dysfunction and oxidative stress following a meal rich in advanced glycation end-products in individuals with type 2 diabetes.⁶⁷ Benfotiamine prevented the endothelial dysfunction that was caused by the ingestion of a meal with high AGE content in both the micro- and macrocirculation, also preventing the increase in biochemical markers of endothelial dysfunction and oxidative stress. In a recent study, benfotiamine administration in diabetic patients with polyneuropathy for a three-week period resulted in a significant decrease in neuropathic pain without any significant adverse effects.⁶⁷ In summary, the above studies indicate that benfotiamine may have a beneficial effect, reducing cardiovascular risk in diabetes. However, its use cannot be recommended on the basis of the existing data as large clinical trials with clinical end-points are required before any recommendations can be made.

α -lipoic Acid

α -lipoic acid is a hydrophilic antioxidant, which allows it to exert beneficial effects in both aqueous and lipid cellular environments.



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α -lipoic acid is reduced to its conjugate base, dihydrolipoate, which is able to regenerate other antioxidants such as vitamins C and E, as well as reducing glutathione.

Diabetic animal models demonstrate improvements in metabolic profile and the microvasculature after long-term treatment with α -lipoic acid. These include improvements in blood glucose, plasma insulin, cholesterol, triglycerides, and lipid peroxidation, as well as increased antioxidant enzymatic activity (catalase and glutathione peroxidase activity).⁶⁸ In contrast, short-term treatment with α -lipoic acid in rat models of insulin resistance and insulin deficiency did not improve hyperglycemia or fasting triglycerides.⁶⁹

In randomized controlled human clinical trials, α -lipoic acid has been mainly studied for the treatment of diabetic polyneuropathy. In initial studies, short-term intravenous α -lipoic acid for 19 days improved symptoms⁷⁰ and longer-term α -lipoic acid (initial intravenous infusions, then oral treatments for two years) were demonstrated to objectively improve peripheral nerve function.⁷¹ However, the largest and latest trial followed patients for seven months and demonstrated no improvements in symptoms in the treated group.⁷² In conclusion, long-term studies demonstrate minimal benefits associated with α -lipoic acid supplementation.

Antioxidants and Diet

In 1996, a study in 34,486 post-menopausal women reported that although vitamin E supplementation did not affect the risk of death from cardiovascular disease, increased intake of vitamin E through diet was associated with decreased risk of death from coronary artery disease.⁷³ This study exemplifies the paradox noted in several large-scale clinical and epidemiologic studies that diet but not vitamin supplementation seems to improve cardiovascular outcomes. As diet is beyond the scope of the current review, the efficacy of diet antioxidants will be reviewed briefly.

Mediterranean Diet

Although it may be challenging to fully define the Mediterranean diet given that it is consumed in more than 15 countries that border the Mediterranean Sea, extensive work over the last few decades has shown that this type of diet has impressive effects in reducing cardiovascular risk.⁷⁴ Olive oil, a main component of the diet, has considerable antioxidant properties and is considered to be among the primary factors that contribute to these beneficial effects.⁷⁵ In a recent study involving subjects

with metabolic syndrome, the diet presented anti-inflammatory and antithrombotic properties, improving endothelial function and insulin sensitivity.⁷⁶ Therefore, the current consensus is that a diet that encompasses the main components of the Mediterranean diet can greatly reduce cardiovascular risk in diabetic patients.

Green Tea and Coffee

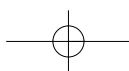
Separate food ingredients and beverages that may have antioxidant effects have also been studied across different populations to examine whether they affect health, and cardiovascular disease in particular. Coffee, a common beverage in Western countries, may have antioxidant effects through minerals (such as magnesium), phytochemicals (in caffeine), and antioxidants. Several studies have shown that coffee decreases the risk of type 2 diabetes, although there have been reports that caffeine itself may impair glucose metabolism in type 2 diabetics.^{77,78} It is not clear, however, how coffee decreases the risk of type 2 diabetes, especially since caffeine (and its phytochemicals) do not seem to play a large role.

Green tea, another widely consumed beverage, also seems to have protective effects because its polyphenols have antioxidant properties. A recent large study that followed Japanese subjects for 11 years found that green tea consumption was associated with a decrease in all-cause mortality and mortality from cardiovascular disease.⁷⁹ In another recently published study, consumption of green tea, coffee, and total caffeine among Japanese subjects was associated with a decreased risk for type 2 diabetes in a five-year follow-up period.⁸⁰

Conclusions

Although there was initially much enthusiasm for antioxidant therapy in diabetes, especially in the form of supplemental vitamins, clinical trials have not shown decreased risk of cardiovascular outcomes. Furthermore, some studies have suggested detrimental effects of vitamins, especially vitamin E. Vitamin E and C supplementation, therefore, cannot be currently recommended. The same can be said for other antioxidants such as tetrahydrobiopterin and benfotiamine, as there are no adequate data from large clinical trials despite initial encouraging preliminary reports. On the other hand, a diet rich in antioxidants, especially Mediterranean diet, can provide considerable reduction of cardiovascular risk and may be of particular benefit to subjects with the metabolic syndrome and/or diabetes. ■

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