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Antioxidants in diabetic complications and insulin resistance

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Introduction

Increased oxidative stress is associated with a variety of pathological conditions including diabetes, atherosclerosis and cardiovascular disease, and neurodegenerative diseases.¹⁻³ Oxidative stress is likely to play a causative role in the tissue and cellular damage in these diseases.^{1,2} In particular, diabetes mellitus is strongly associated with increased oxidative stress, which could be a consequence of either increased production of free radicals, or reduced antioxidant defenses.^{2,4}

In both Type 1 and Type 2 diabetes, late diabetic complications in nerve, vascular endothelial, and kidney arise from chronic elevations of glucose and other metabolites including free fatty acids (FFA). Recent evidence suggests that chronic activation of common stress-activated signaling pathways such as transcription factor nuclear factor- κ B (NF- κ B), p38 MAP kinase (MAPK), and the NH₂-terminal Jun kinases (JNK/SAPK) plays a major role in the etiology of these late diabetic complications. In addition, in Type 2 diabetes, there is evidence that the activation of these same stress pathways by glucose and FFA leads to both impaired insulin secretion and insulin resistance. Thus, we propose a unifying hypothesis whereby hyperglycemia and FFA-induced activation of NF- κ B, p38 MAPK, and

JNK/SAPK stress pathways, along with the additional stress pathways, plays a key role in causing late complications in Type 1 and Type 2 diabetes, along with insulin resistance and impaired insulin secretion in Type 2 diabetes (Figure 29.1).⁵ Studies with antioxidants such as α -lipoic acid (LA), vitamin E, and others suggest that new strategies may become available to treat these conditions.

Hyperglycemia and oxidative stress

A number of processes have been described to explain how hyperglycemia mediates its toxic effects both intra- and extracellularly.⁶⁻⁹ A widely recurring theme is increased oxidative stress, defined as a persistent imbalance between the production of highly reactive molecular species (chiefly oxygen and nitrogen) and antioxidant defenses, leading to potential tissue damage. Many, though not all, experimental and clinical studies have found evidence of increased oxidative stress in diabetes.^{2,4,10,11} Oxidative stress can result from either increased production of reactive oxygen species (ROS), or their inadequate removal (or both). Examples of ROS include charged species such as superoxide, hydroxyl radical, and uncharged species such as hydrogen peroxide (Table 29.1).

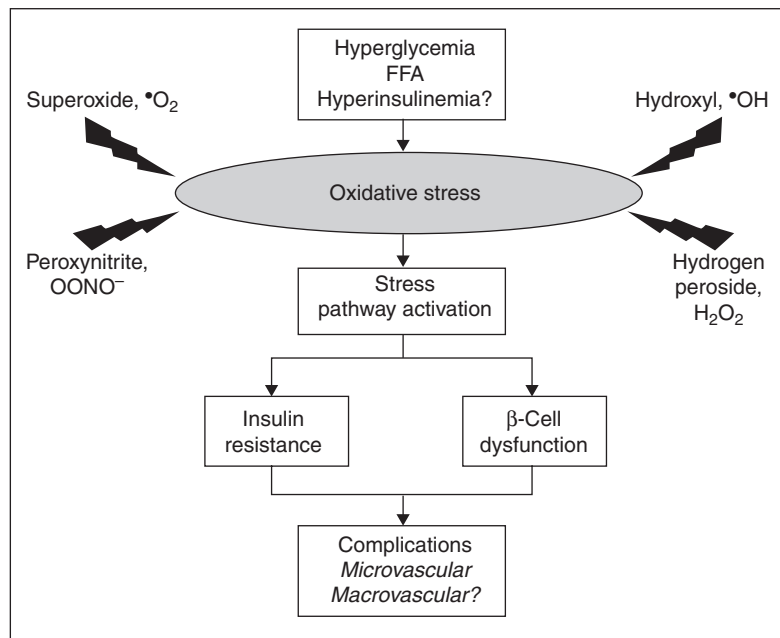


Figure 29.1
 Proposed general theory of how hyperglycemia, elevated free fatty acids, and possibly hyperinsulinemia result in the pathophysiology of diabetes via the generation of reactive oxygen species. This diagram shows the proposed causative link between hyperglycemia, elevated free fatty acids (FFA), reactive oxygen species (ROS) generation, oxidative stress, activation of stress-sensitive pathways (e.g. *NF-κB*, *p38 MAPK*, *JNK/SAPK*, and others), insulin resistance, β-cell dysfunction, and diabetic complications.^{9,27} It is important to note that ROS can inflict damage directly upon cellular macromolecules (not shown), ultimately leading to complications of diabetes. See text for details.

Type	Free-radicals	Non-radicals
Reactive oxygen species (ROS)	Superoxide, $\cdot\text{O}_2^-$ Hydroxyl, $\cdot\text{OH}$ Peroxy, $\cdot\text{RO}_2^-$	Hydrogen peroxide, H_2O_2 Hydrochlorous acid, HOCl
Reactive nitrogen species (RNS)	Hydroperoxyl, $\cdot\text{HO}_2^-$ Nitric oxide, $\cdot\text{NO}$ Nitrogen dioxide, $\cdot\text{NO}_2^-$	Peroxynitrite, OONO^- Nitrous oxide, HNO_2

Notes: Reactive oxygen and nitrogen species (ROS, RNS) are defined as highly reactive molecules including charged species such as superoxide, hydroxyl radical, and nitric oxide and uncharged species such as hydrogen peroxide. *Source:* Adapted from Rösen et al.²

Table 29.1
 Selected examples of biologically important reactive species

There is considerable evidence to show that ROS formation is a direct consequence of hyperglycemia; more recent studies have suggested that ROS formation also results from increased FFA. Excessive production of ROS, or their inadequate neutralization by antioxidants, leads to the damage of proteins, lipids, and DNA.¹² In the absence of an appropriate compensatory response from the endogenous antioxidant network, the system becomes overwhelmed (redox imbalance). A major cellular antioxidant system is reduced glutathione (GSH), which is regenerated by vitamins C and E, LA and other antioxidants (Figures 29.2 and 29.3).^{13,14} LA is a naturally occurring antioxidant and co-factor in the pyruvate dehydrogenase complex, and participates in establishing a cellular antioxidant network by raising intracellular glutathione levels.¹⁵ LA has been shown to:

- (1) quench free-radicals;
- (2) prevent singlet oxygen induced DNA damage;
- (3) chelate metals;
- (4) reduce lipid peroxidation;
- (5) increase intracellular glutathione levels; and
- (6) prevent glycation of serum albumin.^{13,14}

When the endogenous antioxidant network fails to provide a sufficient compensatory response to restore cellular redox balance, oxidative stress ensues.

In addition to their ability to directly inflict damage upon cellular macromolecules, ROS play a significant role in regulating gene expression. ROS activate multiple stress-sensitive intracellular signaling pathways such as NF- κ B, p38 MAPK, JNK/SAPK, PKC, AGE/RAGE, sorbitol, and others. The consequence is the production of an array of gene products¹⁴ that, in turn, cause cellular damage

and are ultimately responsible for the late complications of diabetes.

Hyperglycemia and stress-activated pathways

Hyperglycemia activates several major well characterized biochemical pathways that play a significant role in the development of diabetic complications, including AGE/RAGE,¹⁷ PKC γ , and the polyol pathway.¹⁸ More recently, hyperglycemia has been implicated in the activation of additional biochemical pathways that appear to promote the development of the late complications of diabetes, along with exerting a negative influence on insulin action and insulin secretion. These pathways include the hexosamine pathway,^{19–22} and the stress-activated signaling pathways including NF- κ B, JNK/SAPK, and p38 MAP kinase pathways.^{23,24}

The most extensively studied intracellular target of hyperglycemia and oxidative stress is the transcription factor nuclear factor- κ B (NF- κ B).²⁵ NF- κ B plays a critical role in mediating immune and inflammatory responses, and apoptosis. NF- κ B regulates the expression of a large number of genes, including growth factors (e.g. vascular endothelial growth factor; VEGF), pro-inflammatory cytokines (e.g. TNF- α , IL-1 β), the receptor for advanced glycation end products (RAGE), adhesion molecules (e.g. VCAM-1), and others. Many products of the genes regulated by NF- κ B also, in turn, activate NF- κ B (e.g. VEGF, TNF- α , IL-1 β , RAGE). The aberrant regulation of NF- κ B is associated with a number of chronic diseases including diabetes and atherosclerosis. NF- κ B is activated through a common pathway, which involves the phosphorylation-induced, proteasome-mediated degradation of the inhibitory subunit, I κ B.²⁶ I κ B is phosphorylated by the upstream serine kinase, IKK β , which is phosphorylated and activated by additional upstream serine kinases.

Hyperglycemia leads to mitochondrial dysfunction and activation of NF- κ B and other stress pathways

Compelling evidence demonstrating the importance of ROS generation in mediating hyperglycemia-induced cellular damage was recently provided.²⁷ In bovine endothelial cells, exposure to hyperglycemia initially increased the production of intracellular ROS and activated NF- κ B. Subsequently, PKC activity, AGE, and sorbitol levels increased. Disruption of mitochondrial ROS production was achieved using several different approaches including:

- (1) treatment with CCCP (carbonyl cyanide m-chlorophenylhydrazone), a small molecule uncoupler of mitochondrial oxidative phosphorylation;
- (2) overexpression of UCP-1, a protein uncoupler; or
- (3) overexpression of manganese superoxide dismutase, the mitochondrial antioxidant enzyme.

Each of these approaches blocked the hyperglycemia-induced increase in ROS production. As a consequence, the hyperglycemia-induced effects on NF- κ B, PKC, AGEs, and sorbitol were also suppressed. Moreover, the effects of hyperglycemia on ROS formation and NF- κ B activation preceded the stimulation of the other systems, indicating that activation of NF- κ B was an initial and crucial signaling event leading to the activation of additional stress-sensitive pathways (Figure 29.1).

Hyperglycemia-dependent NF- κ B activation in patients with diabetes mellitus

When patients with diabetes mellitus were studied, a positive correlation of NF- κ B

activation in peripheral blood mononuclear cells was found with the quality of glycemic control (indicated by HbA1c).^{28,29} Moreover, a significant correlation between mononuclear NF- κ B binding activity and the severity of albuminuria was observed in diabetic patients with renal complications.²⁹ When patients with diabetes were treated with the antioxidant LA, a significant suppression of NF- κ B activation as well as of plasma markers for lipid oxidation was observed.^{28,29} These observations further support the idea that hyperglycemia-induced late diabetic complications result from a cycle of oxidative stress-mediated cellular damage, which further exacerbates the condition of increased oxidative stress.

Antioxidants and complications of diabetes

In addition to an increase in reactive ROS, a decrease in antioxidant capacity occurs in diabetes mellitus.² A decline in important cellular antioxidant defense mechanisms, including the glutathione redox system, vitamin C – vitamin E cycle, and the LA/dihydrolipoic acid (DHLA) redox pair (Figures 29.2 and 29.3), significantly increases susceptibility to oxidative stress. Thus, attempts have been made to reduce oxidative stress-dependent cellular changes in patients with diabetes by supplementation with naturally occurring antioxidants, especially LA, vitamin E, and vitamin C.^{30–34} The major goals of antioxidant treatment have been to reduce oxidative stress with the expectation of:

- (1) preventing;
- (2) delaying the progression; or
- (3) reversing (i.e. improving) the late microvascular and/or macrovascular complications of diabetes.

α -Lipoic acid

In patients with diabetes, LA levels are reduced.^{14,35} LA has been prescribed in Germany for over 30 years for the treatment of diabetic neuropathy, a major microvascular complication of diabetes.³⁰ There have been four recent controlled clinical studies evaluating LA for the treatment of diabetic neuropathy, and one study for the treatment of autonomic neuropathy. The overall conclusions are:

- (1) three-week treatment with i.v. LA (600 mg) reduced the main symptoms of diabetic polyneuropathy;
- (2) the effect is accompanied by an improvement in neuropathic deficits;
- (3) oral treatment with LA (800–1800 mg) for four to seven months appears to improve neuropathic deficits and autonomic neuropathy;
- (4) preliminary data also suggest an improvement in motor and sensory function in lower limbs;
- (5) LA has an excellent safety profile at oral doses up to 1800 mg/day.

A pivotal multicenter trial, NATHAN (Neurological Assessment of Thioctic Acid in Neuropathy) Study, is in process in Europe and North America to evaluate the ability of oral LA to slow the progression of diabetic neuropathy.³⁰ This study is using the most rigid statistical design and quantitative indices of efficacy of any trial performed to date. If efficacy is demonstrated, LA could become the first approved treatment for diabetic neuropathy in the USA. Results from a recent study indicated that treatment with LA improved endothelial function in patients with diabetes.³⁶

Vitamin E

Cardiovascular disease is the leading cause of

morbidity and mortality in the Western world, and the major macrovascular complication of diabetes.³⁷ It is associated with increased oxidative stress,³ and studies, both *in vitro* and *in vivo* have provided the rationale for numerous prospective clinical studies evaluating the effects of vitamin E (α -tocopherol) on cardiovascular events in different populations.^{38,39} A review of these data by Jialal and colleagues has led to the overall conclusion that four of the five major prospective trials have reported a beneficial effect on cardiovascular end points, including cardiovascular death, non-fatal myocardial infarction, ischemic stroke, peripheral vascular disease, and others.³⁹ The one major study (HOPE Study⁴⁰) that was negative for all end points had several limitations.³⁹ It was terminated early due to the overwhelming positive effects of the angiotensin-converting enzyme ramipril, its lack of data on the dietary intake of other antioxidants, and its evaluation of synthetic vitamin E (a mixture of tocopherols and tocotrienols) and not α -tocopherol, the most potent and effective tocopherol. In a study in patients with Type 2 diabetes evaluating the effects of vitamin E on biochemical risk factors for the development of cardiovascular disease, vitamin E treatment significantly reduced low-density lipoprotein oxidizability and soluble cell adhesion molecules.⁴¹ Taken together the evidence suggests a beneficial effect of vitamin E in patients with pre-existing cardiovascular disease, and in those who are at a greater risk for its development.

Oral vitamin E treatment appears to be effective in normalizing abnormalities in retinal hemodynamics, and improving renal function in patients with Type 1 diabetes of short (disease) duration.³¹ Vitamin E was beneficial in those individuals with poorest glycemic control and the most impaired retinal blood flow (Figure 29.4).³¹ In a well controlled study,

short-term (four weeks) supplementation of patients with Type 2 diabetes with persistent micro/macrolalbuminuria with both vitamins E and C significantly lowered their urinary albumin excretion rate.⁴² Four months' treatment of patients with Type 2 diabetes with autonomic neuropathy with vitamin E improved the ratio of cardiac sympathetic to parasympathetic tone coincident with lowering of several indices of oxidative stress.⁴³ Interestingly, the study also reported a lowering of glycated hemoglobin, insulin, nor-epinephrine, and the homeostatic model assessment index, indicative of increased insulin sensitivity and glycemic control. These data suggest that vitamin E and perhaps other antioxidant supplementation may provide a benefit in the treatment of microvascular complications of diabetes including diabetic retinopathy or nephropathy.

Vitamin C

The normal functions of vascular endothelial tissue include regulation of vasomotor tone, inhibition of platelet activity, and regulation of recruitment of inflammatory cells into the vasculature.⁴⁴ A damaged endothelium ('endothelial dysfunction') is a key event in the development of diabetic macroangiopathy, and is associated with the oxidative stress-mediated blunting of nitric oxide action.^{45,46} Endothelial dysfunction has been documented in individuals who are insulin-resistant, and in those at risk for developing Type 2 diabetes.⁴⁷ Acute treatment with vitamin C improved endothelial function in obese subjects,⁴⁸ and in patients with Type 1, Type 2, and gestational diabetes.⁴⁹⁻⁵¹

In patients with cardiovascular disease, including endothelial dysfunction, both acute (single dose, 2 g) and chronic treatment with vitamin C (30 days, 500 mg/d) reverses the vasomotor defect, as judged by increased

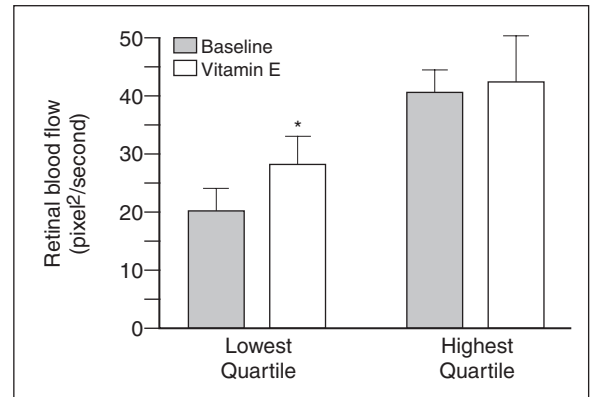


Figure 29.4

*Vitamin E treatment increases retinal blood flow in patients with Type 1 diabetes. An eight-month randomized double blind placebo-controlled crossover trial evaluated 36 patients with Type 1 diabetes and nine non-diabetic control subjects.³¹ Subjects were randomly assigned to receive either 1800 IU vitamin E/day or placebo for four months and followed, after treatment crossover, for an additional four months. Retinal blood flow (RBF) was measured at baseline, and at months four and eight using video fluorescein angiography (details of procedure provided in reference). In diabetic patients at baseline, RBF (29.1 ± 7.5 pixel²/s) was significantly ($p < 0.03$) reduced compared with non-diabetic controls (35.2 ± 6.4 pixel²/s). After four months' treatment with vitamin E, RBF (34.5 ± 7.8 pixel²/s) in patients with diabetes was significantly increased ($p < 0.002$), and normalized compared with non-diabetic controls. Subsequent analyses were performed on diabetic patients grouped into quartiles based on baseline RBF. Patients in the lowest quartile (shown above) exhibited the largest increase in RBF (9.4 ± 3.2 pixel²/s) after treatment with vitamin E (19.9 ± 3.5 to 28.9 ± 3.0 pixel²/s). The increases in RBF in response to vitamin E treatment were progressively less in the higher quartiles (quartile 2, 6.0 ± 6.6 pixel²/s, $p < 0.03$ (data not shown); quartile 3, 6.0 ± 6.1 pixel²/s, $p < 0.02$) (data not shown); and quartile 4 (highest; shown above), 1.1 ± 5.3 pixel²/s, not significant). Notes: *, $p < 0.003$ (compared to baseline). All values are mean \pm SD. Source: figure redrawn with modifications from Bursell S-E et al.³¹*

flow-mediated dilation of the brachial artery.^{52,53} All of the above studies involved relatively small populations (<75) and used acute treatment except one, which was for 30 days.⁵³ Nonetheless, the persistent finding of a beneficial effect of antioxidant treatment on endothelial function (flow-mediated dilation) in individuals with demonstrated endothelial dysfunction is encouraging. It is likely that these results will stimulate additional clinical studies of larger size and longer duration to evaluate the efficacy of vitamin C and perhaps other antioxidants.

Antioxidants and insulin resistance

Oxidative stress not only is associated with complications of diabetes, but also has been linked to insulin resistance *in vitro* and *in vivo*.^{54–58} Both insulin resistance and decreased insulin secretion are major features of Type 2 diabetes.^{59,60} Insulin resistance most often precedes the onset of Type 2 diabetes by many years, is present in a large segment of the general population, and is multifactorial.^{59,60} Clearly, insulin resistance has a genetic component.^{59,61,62} Insulin resistance also is caused by acquired factors such as obesity, sedentary lifestyle, pregnancy, and hormone excess.⁵⁹ Initially, insulin resistance is compensated for by hyperinsulinemia, thus preserving normal glucose tolerance. Reaven and others have presented data that show at least 25% of non-diabetic individuals have insulin resistance that is in the range of that seen in patients with Type 2 diabetes.⁶⁰ Deterioration into impaired glucose tolerance occurs when either insulin resistance increases or the insulin secretory responses decrease, or both.

When glucose and FFA increase, they cause oxidative stress along with activation of

stress-sensitive signaling pathways. Activation of these pathways, in turn, worsens both insulin action and secretion leading to overt Type 2 diabetes.⁶³ Furthermore, insulin-resistant patients, with and without Type 2 diabetes, are at increased risk for developing the Metabolic Syndrome, a major cause of heart disease, hypertension and dyslipidemia.⁶⁰ Thus, treatment aimed at reducing the degree of oxidative stress and activation of oxidative stress signaling pathways would appear to warrant consideration for inclusion as part of the treatment program for patients with Type 2 diabetes.

Antioxidants and insulin sensitivity: clinical studies

α -Lipoic acid

Studies in animal models of diabetes indicate that antioxidants, especially LA, improve insulin sensitivity.⁶⁴ A number of studies have found that the antioxidants LA, glutathione, vitamin E, and vitamin C increase insulin sensitivity in patients with insulin resistance, Type 2 diabetes, and/or cardiovascular disease. In patients with Type 2 diabetes, both acute and chronic administration of LA improves insulin resistance as measured by both the euglycemic-hyperinsulinemic clamp and the Bergman minimal model (Figure 29.5).^{65–70} In addition, the short-term (six-week) oral administration of a novel controlled release formulation of LA lowered plasma fructosamine levels in patients with Type 2 diabetes.⁷¹

Glutathione

In patients with Type 2 diabetes, there is a significant inverse correlation between fasting plasma FFA concentration and the ratio of reduced/oxidized glutathione (a major endogenous antioxidant).⁵⁷ In healthy subjects, infusion of FFA (as Intralipid) causes increased

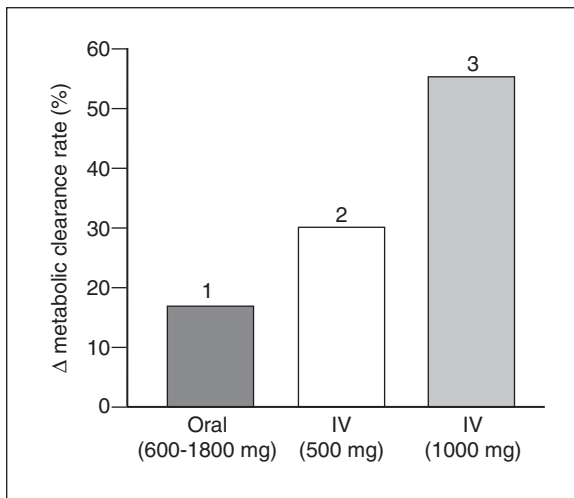


Figure 29.5

α-Lipoic acid increases insulin-stimulated glucose metabolism in patients with Type 2 diabetes. Oral and intravenous (IV) administrations of α-lipoic acid are able to significantly increase insulin sensitivity (as judged by % change (Δ) in metabolic clearance rate (MCR)) in patients with Type 2 diabetes. Effect is greater after IV administration. Each study employed the euglycemic-hyperinsulinemic clamp for assessment of insulin sensitivity.⁷⁰ (1) Oral administration of LA enhanced insulin-stimulated glucose disposal in patients with Type 2 diabetes.⁶⁷ In a randomized, placebo-controlled, multicenter study, LA (600, 1200, or 1800 mg per day) was administered to 74 patients with Type 2 diabetes for four weeks. Subjects were well controlled by diet alone, or diet combined with

*other antihyperglycemic medications. Subjects in each arm of the study had a similar degree of hyperglycemia and insulin sensitivity at baseline. Compared to the placebo group, a greater percentage of patients who received LA treatment exhibited an increase in MCR (insulin sensitivity). No differences were observed among groups receiving the different doses of LA. Thus, patients from each arm were combined into a single group for comparison with those who received the placebo tablet. Overall, insulin sensitivity improved approximately 17% following LA treatment ($p < 0.05$). Fasting plasma glucose did not change, but there was a trend toward reduced fasting insulin. (2) Repeated parenteral administration of 500 mg LA (daily infusions for ten days) enhanced insulin-stimulated glucose disposal in patients with Type 2 diabetes.⁶⁶ Study subjects ($n = 20$) were well controlled by diet alone, or diet combined with glibenclamide and/or acarbose. After treatment with LA for 10 days, MCR (insulin sensitivity) was significantly increased by approximately 30% ($p < 0.05$). (3) Acute intravenous infusion of 1000 mg of LA significantly improved insulin-stimulated metabolic clearance rate (MCR) and insulin sensitivity in patients with Type 2 diabetes.⁶³ Study subjects ($n = 13$) were well controlled by diet alone, or diet combined with glibenclamide. After LA treatment, the glucose infusion rate increased 47% ($p < 0.05$), and MCR increased 55% ($p < 0.05$). No improvement was seen in the saline-treated control group. Source: figure reprinted with permission from Evans JL, et al. *Diabetes Technol Therapeut* 2000; 2: 401–13.⁶⁹*

oxidative stress as judged by increased malondialdehyde levels and a decline in the plasma reduced/oxidized glutathione ratio.⁵⁸ Malondialdehyde, a highly toxic by-product generated in part by lipid oxidation and ROS, is increased in diabetes mellitus.⁷² In both healthy individuals and in subjects with Type 2 diabetes, restoration of redox balance by infusing glutathione improves insulin sensitivity along with β-cell function.⁷³

Vitamin E

Initial reports of a positive effect of vitamin E on insulin action in insulin-resistant patients with Type 2 diabetes were published almost 10 years ago.^{74,75} Twenty-five patients with Type 2 diabetes were treated with vitamin E (d-α-tocopherol; 900 mg/d) or placebo for three months in a double blind, crossover design.⁷⁵ There was a trend in the reduction of plasma glucose, along with significant reductions in

HbA1c levels (7.8 versus 7.1), triglycerides, free fatty acids, total cholesterol, low-density lipoprotein cholesterol, and apoprotein B.⁷³ The β -cell response to glucose was unaffected. These intriguing results prompted additional evaluations by Paolisso and colleagues using a more sensitive technique to measure insulin sensitivity, the euglycemic-hyperinsulinemic clamp.

Ten healthy subjects and 15 patients with Type 2 diabetes underwent an oral glucose tolerance test and euglycemic-hyperinsulinemic clamp before and after vitamin E supplementation (900 mg/d for 4 mo).⁷⁶ In patients with Type 2 diabetes, vitamin E supplementation significantly increased both whole-body glucose disposal (i.e. insulin sensitivity) by approximately 50%, and non-oxidative glucose disposal by approximately 60%. Vitamin E also improved insulin action in the healthy subjects.

Vitamin E also improved insulin action in elderly people.⁷⁷ Twenty elderly, non-obese subjects with normal glucose tolerance were submitted to the euglycemic-hyperinsulinemic clamp in a double blind, crossover, and randomized study after four months' treatment with either vitamin E (900 mg/d) or placebo. Whole-body glucose disposal was significantly potentiated by vitamin E compared to placebo. Furthermore, plasma vitamin E concentrations were correlated with net changes in insulin-stimulated whole-body glucose disposal.

In a four-week, double blind, randomized study of vitamin E administration (600 mg/d) versus placebo in 24 hypertensive patients, whole-body glucose disposal was measured by the euglycemic-hyperinsulinemic clamp.⁷⁸ In hypertensive subjects, vitamin E administration significantly increased whole-body glucose disposal, along with the ratio of reduced glutathione/oxidized glutathione in plasma.

Four months' treatment of patients with Type 2 diabetes with cardiac autonomic

neuropathy with vitamin E lowered glycated hemoglobin, insulin, norepinephrine, and the homeostatic model assessment index, was indicative of increased insulin sensitivity and improved glycemic control.⁴³

Vitamin C

In addition to playing a major role in the etiology of diabetic macroangiopathy, endothelial dysfunction could promote insulin resistance.⁴⁷ It is possible that oxidative stress-mediated blunting of nitric oxide action indirectly affects insulin sensitivity (e.g. reduced peripheral blood flow, increased peroxynitrite formation) consequently reducing insulin-stimulated glucose transport in skeletal muscle.

Cigarette smoking impairs endothelial function, and is one of the major risk factors for hypertension, atherosclerosis, and coronary heart disease. The effects of vitamin C (infusion) on insulin sensitivity and endothelial function (measured by flow-mediated dilation of brachial artery; FMD) were evaluated in smokers, non-smokers with impaired glucose tolerance, and non-smokers with normal glucose tolerance.⁷⁹ Both insulin sensitivity and FMD were blunted in smokers and non-smokers with IGT, compared with controls. In smokers and in non-smokers with impaired glucose tolerance, vitamin C significantly improved FMD, increased insulin sensitivity, and decreased plasma thiobarbituric acid-reactive substances, an index of oxidative stress. In contrast, vitamin C had no effect on these parameters in non-smokers with normal glucose tolerance. In patients with coronary spastic angina and endothelial dysfunction, vitamin C infusion augmented FMD and increased insulin sensitivity.⁸⁰ In contrast, vitamin C had no effect in healthy controls.

It is important to note that these trials have been relatively small and of short duration. Although consistent and very encouraging, these

results need to be confirmed in larger, double blind, placebo-controlled studies. Ideally, these trials would include the measurement of multiple indices of oxidative stress, plasma levels of antioxidants, and measures of insulin sensitivity and glycemic control. Nonetheless, the beneficial effects on insulin action reported following treatment with the antioxidants LA, vitamin E, and vitamin C clearly support the idea that there is an interaction between oxidative stress and insulin action. This area of research certainly merits further and more detailed investigation, with a particular focus on identifying molecular mechanisms along with the sites of antioxidant action.

Antioxidants and insulin sensitivity: possible sites of action

Protection against inhibition of insulin signal transduction mediated by activation of stress-kinases and increased serine phosphorylation

As discussed previously, oxidative stress leads to the activation of multiple serine kinase cascades, including p38 MAPK, JNK/SAPK, and IKK β /NF- κ B.^{81–83} There are a number of potential targets of these kinases in the insulin signaling pathway, including the insulin receptor and the insulin receptor substrate (IRS) family of proteins. Increased phosphorylation of the insulin receptor or IRS on discrete serine or threonine sites decreases the extent of their tyrosine phosphorylation, and is consistent with impaired insulin action including protein kinase B activation, and glucose transport.^{84–89} There is growing evidence *in vitro* that activation of each of these pathways can render cells insulin resistant. When activation of these pathways is prevented, insulin action is restored (Figure 29.6).

Recently, it has been reported that activation of IKK β , which activates NF- κ B, inhibits

insulin action.⁹⁰ Salicylates, which inhibit IKK β activity,⁹¹ restore insulin sensitivity both *in vitro* and *in vivo*.^{90,92} Treatment with aspirin and salicylates alter the phosphorylation patterns of the IRS proteins, resulting in decreased serine phosphorylation and increased tyrosine phosphorylation.^{90,92} Preliminary clinical evidence implicating IKK β in insulin resistance has also been recently provided. Treatment of nine patients with Type 2 diabetes for two weeks with high-dose aspirin (7 g/day) resulted in reduced hepatic glucose production, fasting hyperglycemia, and increased insulin sensitivity.⁹³ Taken together, these data support a role for activation of IKK β and NF- κ B in the pathogenesis of insulin resistance, and suggest that it might be an attractive pharmacological target to increase insulin sensitivity.

Antioxidants and prevention of NF- κ B activation in vitro

Activation of NF- κ B *in vitro* can be blocked by several thiol-containing antioxidants including LA,⁹⁴ a positively charged analog of LA with increased potency,⁹⁵ N-acetyl-L-cysteine (NAC),⁹⁶ and the glutathione precursor L-2-oxothiazolidine-4-carboxylic acid.⁹⁷ Other clinically available antioxidants reported to have anti-inflammatory, antioncogenic, and/or antiatherogenic properties that have been shown to block the activation of NF- κ B include resveratrol,^{97,99} (-)-epicatechin-3-gallate,¹⁰⁰ pycnogenol,¹⁰¹ silymarin,¹⁰² and curcumin.¹⁰³ IRFI-042, a novel vitamin E analog, inhibited the activation of NF- κ B, and reduced the inflammatory response in myocardial ischemia-reperfusion injury.¹⁰⁴ Melatonin is another example of an antioxidant that inhibits NF- κ B activation.¹⁰⁵

Alpha-phenyl-tert-butyl nitron (PBN), an effective spin-trapping agent that reacts with and stabilizes free-radical species,¹⁰⁶ significantly reduced the severity of hyperglycemia in

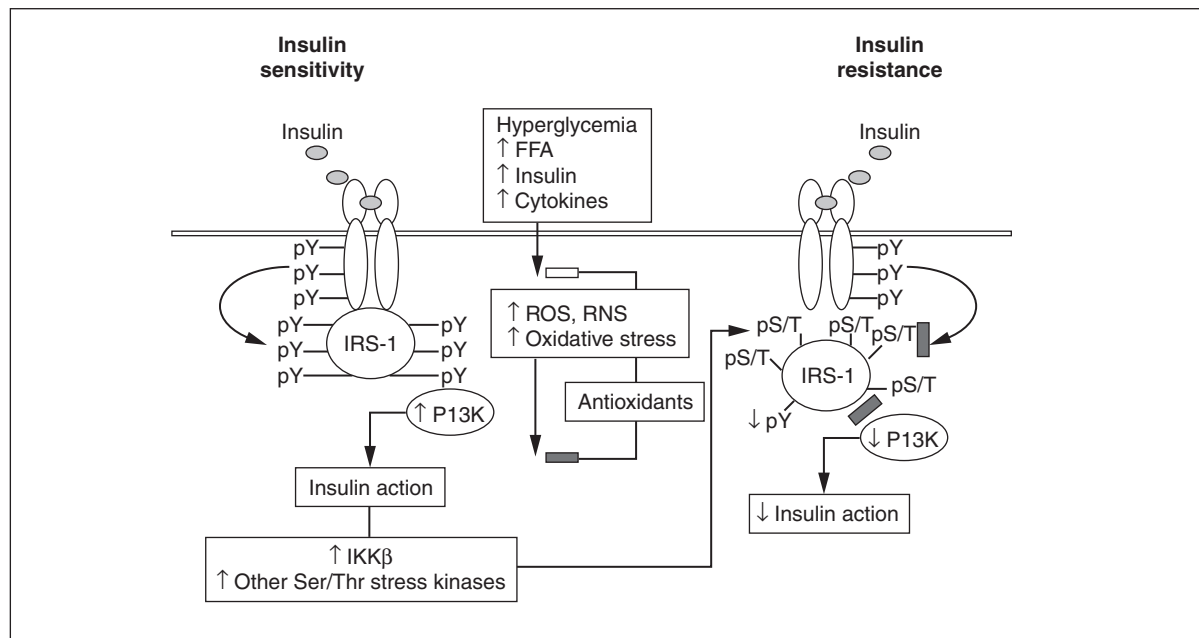


Figure 29.6

Possible sites of action to account for the protective effects of antioxidants against oxidative stress-induced insulin resistance. A variety of stimuli including hyperglycemia, elevated free fatty acids (FFA), hyperinsulinemia, cytokines, and other agents increase ROS production and oxidative stress. This results in the activation of multiple stress-sensitive serine/threonine kinase signaling cascades such as IKK β and others. Once activated, these kinases are able to phosphorylate multiple target proteins including the insulin receptor and the insulin receptor substrate (IRS) proteins. Increased phosphorylation of the insulin receptor or IRS proteins on discrete serine or threonine sites (pS/T) decreases the extent of their tyrosine phosphorylation (pY).⁸²⁻⁸⁷ Consequently, the association and/or activities of downstream signaling molecules (e.g. phosphatidylinositol 3-kinase; PI3K) are decreased resulting in decreased insulin action (insulin resistance).⁸⁵⁻⁸⁷ The protective effects of α -lipoic acid (LA) and other antioxidants on oxidative stress-induced insulin resistance may relate to their ability to preserve the intracellular redox balance (neutralizing ROS), or by directly preventing the activation of redox-sensitive kinases (e.g. IKK β). Source: reprinted with modifications and permission from Evans et al. *Diabetes* 2002; in press.⁶³

both alloxan- and streptozotocin (STZ)-induced diabetes, coincident with inhibiting both alloxan- and STZ-induced activation of NF- κ B.¹⁰⁷ Inhibiting the activation of NF- κ B prevents the activation and the transcription of genes under NF- κ B control, including VEGF and others.¹⁰⁸ These studies are consistent with the suggestion that one site of action of

antioxidants might be in the NF- κ B pathway. With the exception of LA, little attention has been afforded other antioxidants that block NF- κ B activation with respect to their potential impact on insulin action. An important goal of future studies in this area will be the determination of which antioxidants are the most effective at preventing NF- κ B activation,

whether they affect insulin action, and the identification of their molecular sites of action.

Conclusions and implications

The molecular mechanisms whereby oxidative stress causes diabetic complications are undefined. In a variety of tissues, hyperglycemia and elevated FFA result in the generation of ROS and RNS, leading to increased oxidative stress. In the absence of an appropriate compensatory response from the endogenous antioxidant network, the system becomes overwhelmed (redox imbalance), leading to the activation of stress-sensitive signaling pathways, such as NF- κ B, p38 MAPK, JNK/SAPK, PKC, AGE/RAGE, sorbitol, and others. The consequence is the production of gene products such as VEGF and others that cause cellular damage, and are ultimately responsible for the long-term complications of diabetes. In addition, activation of the same or similar pathways appears to mediate insulin resistance and impair insulin secretion. It is our view that there appears to be a common biochemical basis that involves oxidative stress-induced activation of stress-sensitive signaling pathways. Thus, the use of antioxidants may be very important in preventing activation of these pathways. Moreover, identification of the molecular basis for the protection afforded by a variety of antioxidants against oxidative-induced damage might lead to the discovery of pharmacological targets for novel therapies to prevent, reverse, or delay the onset of the resultant pathologies.

Acknowledgements

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