Antioxidants: Do they have a role in the treatment of insulin resistance?

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Insulin resistance, defined as an attenuated or inadequate response to a given amount of insulin, is associated with a wide variety of conditions including obesity, type 2 diabetes, essential hypertension, cardiovascular disease, polycystic ovary syndrome, non-alcoholic fatty liver, breast cancer, and acquired immune deficiency syndrome. Although pharmacological options for the management of insulin resistance and type 2 diabetes have been increasing, not all patients benefit, as the cost of prescription medications is often beyond the financial capacity of many patients. A potential new approach is the use of antioxidants. The objectives of this review are to discuss the scientific rationale for proposing the evaluation of antioxidants for insulin resistance, and to provide an update of intervention studies, with an emphasis on clinical trials, in which antioxidants have been tested. Briefly, this approach capitalizes on emerging data implicating lipid oversupply, chronic, low-grade inflammation, and oxidative stress as root causes in the development and exacerbation of insulin resistance.

Key words Antioxidants - diabetes - insulin resistance - lipoic acid

Insulin resistance, defined as an attenuated or inadequate response to a given amount of insulin, is associated with a wide variety of conditions including obesity, type 2 diabetes (T2D), essential hypertension, cardiovascular disease, polycystic ovary syndrome, non-alcoholic fatty liver, breast cancer, and acquired immune deficiency syndrome (AIDS)\textsuperscript{1-3}. An individual with insulin resistance is strongly predisposed to an increased risk for life threatening clinical conditions including T2D, and the metabolic syndrome\textsuperscript{4}(also known as the insulin resistance syndrome\textsuperscript{5}). T2D has reached epidemic proportions in the US and worldwide (>18 million and 160 million individuals, respectively), and is projected to increase dramatically\textsuperscript{6}. There are an estimated 50 million individuals (US) and 314 million individuals (worldwide) with the metabolic syndrome\textsuperscript{7,8}. This situation is further
exacerbated by obesity, a major risk for developing T2D. The number of adults overweight or obese in the US is 125 million (65% of population) and 1.3 billion worldwide\textsuperscript{6}. In addition, there is an increasing prevalence of adult and childhood obesity that markedly contributes to the development of T2D\textsuperscript{10-12}. Although pharmacological options for the management of insulin resistance and T2D in obese individuals have been increasing\textsuperscript{13,14}, not all patients benefit, as the cost of prescription medications often exceeds the financial capacity of an increasing number of patients\textsuperscript{15,16}.

Insulin resistance has an inherited component\textsuperscript{17} that is exacerbated by environmental factors, including obesity, ageing, hyperglycaemia and oxidative stress\textsuperscript{18-20}. Reaven and co-workers were the first to investigate apparently healthy, non-obese, insulin resistant, non diabetic individuals from the general population\textsuperscript{21,22}. Strikingly, they observed that 25 per cent had insulin resistance that was of a magnitude similar to that seen in patients with T2D, but exhibited compensatory hyperinsulinaemia to maintain normoglycaemia. However, even without developing hyperglycaemia and diabetes, these insulin-resistant individuals pay a significant price in terms of general health. Longitudinal studies have clearly demonstrated that insulin resistance predisposes non diabetic individuals not only to T2D, but also to the metabolic syndrome and even cancer\textsuperscript{22}.

Efforts to identify and develop effective approaches to treat insulin resistance-related disorders have increased dramatically in recent years. The two major non pharmacological approaches to increase insulin sensitivity include weight loss and exercise training. The two major pharmacological approaches are biguanides (metformin) and thiazolidinediones\textsuperscript{23-25}. Although both classes of drugs are efficacious, they are often associated with undesirable side effects, such as gastrointestinal disturbances (metformin), oedema and weight gain (thiazolidinediones)\textsuperscript{24}. A potential new approach is the use of antioxidants\textsuperscript{26}. The objectives of this review are to discuss the scientific rationale for proposing the evaluation of antioxidants for insulin resistance, and to provide an update of intervention studies, with an emphasis on clinical trials, in which antioxidants have been tested. Briefly, this approach capitalizes on emerging data implicating lipid oversupply, chronic, low-grade inflammation, and oxidative stress as root causes in the development and exacerbation of insulin resistance\textsuperscript{14,27}.

Why consider anti-oxidants for insulin resistance?

Oxidative stress is not only associated with complications of diabetes\textsuperscript{28-31}, but has been linked to insulin resistance \textit{in vitro} and \textit{in vivo}\textsuperscript{19,32-37}. Both insulin resistance and decreased insulin secretion are major features of type 2 diabetes\textsuperscript{2,18,38}. 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 multi-factorial\textsuperscript{2,18,38}. Clearly, insulin resistance has a genetic component\textsuperscript{18,39,40}. Insulin resistance also is caused by acquired factors such as obesity, sedentary life style, pregnancy, and hormone excess\textsuperscript{2,18}. Initially, insulin resistance is compensated by hyperinsulinaemia, thus preserving normal glucose tolerance. Facchini and colleagues have presented data that at least 25 per cent of non-diabetic individuals have insulin resistance that is in the range of that seen in patients with type 2 diabetes, predisposing these individuals to a wrath of age-related diseases\textsuperscript{22}. Deterioration into impaired glucose tolerance occurs when either insulin resistance increases or the insulin secretory responses decrease, or both\textsuperscript{2}.
When glucose and free fatty acid (FFA) increase, they cause oxidative stress along with activation of stress-sensitive signaling pathways\textsuperscript{19,32,33}. Activation of these pathways, in turn, worsens both insulin action and secretion leading to overt T2D. 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 dyslipidaemia\textsuperscript{38,41,42}. 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 programme for patients with T2D.

**Oxidative stress and insulin resistance: Possible mechanistic link**

*In vitro*, an increase in reactive molecules can lead to the activation of multiple serine kinase cascades\textsuperscript{43-45}, or inhibition of protein tyrosine phosphatases (PTPases)\textsuperscript{46-48}. A variety of other redox-sensitive molecules also exist\textsuperscript{49,50}. As discussed above, these are normal physiological events designed to mediate signal transduction. Under physiological conditions, the increase in reactive molecules does not necessarily cause oxidative stress, as it is counterbalanced by the endogenous antioxidant network (Fig. 1). However, the chronic and/or increased production of these reactive molecules can lead to oxidative stress.

![Fig. 1. Redox balance and oxidative stress.](image)

Components of the endogenous antioxidant system include various proteins (e.g., thioredoxin, metallothionein) and amino acids (e.g., taurine, L-arginine), low molecular weight (MW) antioxidants (e.g., α-lipoic acid, glutathione, vitamin C, vitamin E), and antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase). Under physiological conditions, a redox balance exists; when the production of reactive molecules exceeds the capacity for their clearance, a redox imbalance occurs. When the imbalance persists, oxidative stress ensues. ROS, reactive oxygen species; RNS, reactive nitrogen species. (Copyright © 2005 Mary Ann Liebert, Fig. adapted from Ref 33 (Antioxid Redox Signal 2005; 7: 1040-52. Reprinted with permission from Mary Ann Liebert)}
molecules or a reduced capacity for elimination can cause oxidative stress, dysregulation in intracellular signaling, and ultimately resulting in a pathological situation including insulin resistance32,51.

The insulin signaling pathway offers a number of potential targets (substrates) of these activated kinases, including the insulin receptor and the family of insulin receptor substrate (IRS) proteins. For IRS-1 and IRS-2, an increase in serine phosphorylation decreases the extent of tyrosine phosphorylation, and is consistent with the attenuation of insulin action52,60 (Fig. 2). The serine/threonine phosphorylated forms of IRS molecules are less able to associate with the IR and downstream target molecules especially PI 3-kinase52,61, resulting in impaired insulin action including protein kinase B (PKB) activation, and glucose transport59,62,63. In addition, the serine/threonine phosphorylated forms of IRS molecules are less able to associate with the IR and downstream target molecules especially PI 3-kinase52,61, resulting in impaired insulin action including protein kinase B (PKB) activation, and glucose transport59,62,63. In addition, the serine/threonine phosphorylated forms of IRS molecules are less able to associate with the IR and downstream target molecules especially PI 3-kinase52,61, resulting in impaired insulin action including protein kinase B (PKB) activation, and glucose transport59,62,63.
threonine phosphorylated forms of IRS molecules are more susceptible to proteasome-mediated degradation.60,64-67.

There are several major stress-sensitive kinases that, when activated, are likely involved in attenuating insulin signaling via effects on IRS proteins. In Chinese hamster ovary cells, both the pro-inflammatory cytokine TNF-α and anisomycin stimulate IRS-1-associated c-Jun N-terminal kinase (JNK)/stress-activated protein kinase (SAPK) activity, resulting in increased serine phosphorylation of IRS-1 catalyzed by JNK/SAPK. Consequently, insulin-stimulated tyrosine phosphorylation of IRS-1 was substantially reduced and insulin action impaired. More recent results have indicated that JNK also functions as a negative regulator during normal insulin stimulation. In mouse embryo fibroblasts, 32D cells, and 3T3-L1 adipocytes JNK activity is increased in response to insulin. As shown in previous studies using TNF-α and anisomycin, insulin-stimulated JNK activity led to increased serine phosphorylation of IRS-1 on Ser-307, resulting in reduced insulin signaling. Thus, it appears that JNK serves as a heterologous inhibitor of insulin action during acute and chronic inflammation and as a feedback inhibitor during insulin stimulation. In vivo, JNK activity is increased in insulin target tissues in multiple rodent models of insulin resistance and obesity. The absence of JNK1 in mice results in decreased adiposity, increased insulin sensitivity, and enhanced insulin signaling, consistent with its role as stress- and inflammation-induced negative regulator of insulin action.

Inhibitor kappa β kinase (IKKB), another major stress-sensitive kinase, controls the activation of NF-kβ and is increased in insulin-resistant muscle from a variety of sources. Activation of IKKB increases IRS-1 serine phosphorylation on Ser-307 and inhibits insulin action; salicylates and ligands for PPARγ, both of which inhibit IKKB activity, restore insulin sensitivity both in vitro and in vivo. Recent work has identified IRS-1 as a direct substrate of IKKB. Treatment with aspirin and salicylates alter the phosphorylation patterns of the IRS proteins, resulting in decreased serine phosphorylation and increased tyrosine phosphorylation and insulin action.

Support for the importance of IKKB in insulin resistance in vivo is provided by results of recent gene knockout experiments in mice. IKKB (+/-) heterozygotes were more insulin sensitive (as judged by increased glucose infusion rate during hyperinsulinaemic-euglycaemic clamp) compared to their normal (+/+) littermates. This improvement in insulin sensitivity was even more dramatic when IKKB (+/-) mice were crossed with insulin resistant ob/ob mice. Preliminary clinical evidence implicating IKKB in insulin resistance has also been recently provided. Treatment of nine patients with T2D for two weeks with high-dose aspirin (7 g/day) resulted in reduced hepatic glucose production and fasting hyperglycaemia, and increased insulin sensitivity.

In L6 muscle cells, activation of p38 mitogen-activated protein kinase (MAPK) by oxidative stress (H₂O₂) is linked to H₂O₂-mediated inhibition of insulin-stimulated glucose transport. Inhibition of insulin signaling was reversed by a specific inhibitor of p38 MAPK. Interestingly, p38 MAPK has been suggested as an activator of the glucose transporter. Due to the existence of multiple isoforms of this enzyme, it is possible that this latter effect is mediated by a different isoform.

Additional stress-sensitive kinases that are reported to be involved in IRS-mediated insulin
resistance include the mammalian target of rapamycin (mTOR)\textsuperscript{64,85}, several isozymes of PKC including PKC\ort{\alpha} and PKC\ort{\theta}, the salt-inducible kinase (SIK2)\textsuperscript{87}, and a novel IRS serine kinase\textsuperscript{88}. Clearly, there is compelling evidence that serine/threonine phosphorylation attenuates insulin signaling by reducing the extent of tyrosine phosphorylation of IRS proteins. There is also evidence that increased serine/threonine phosphorylation accelerates the degradation of IRS-1 protein, which also results in insulin resistance\textsuperscript{66-67}. In addition, it is well documented that these kinases (e.g., JNK, IKK\ort{\beta}, PKC, p38MAPK) are activated in response to a variety of stress-inducers including reactive oxygen species (ROS) and other reactive molecules\textsuperscript{32}. The acute activation of JNK by insulin and subsequent increased phosphorylation of IRS-1 on Ser-307 might serve as a signal to terminate the insulin signal under physiological conditions\textsuperscript{69}. However, chronic activation of JNK and likely other serine/threonine kinases as occurs in response to oxidative stress and inflammation leads to the pathological condition of insulin resistance.

To date only one published study has directly evaluated the effects of oxidative stress on IRS serine phosphorylation and IRS protein content, in the context of cellular insulin resistance\textsuperscript{89}. Consistent with the molecular basis of oxidative stress-induced insulin resistance proposed here, these investigators found that oxidative stress (H\ort{2}O\ort{2}) caused an increase in serine phosphorylation of IRS-1 and IRS-2, decreased content of IRS-1, and insulin resistance in 3T3-L1 adipocytes. However, the following aspects of this study raise questions regarding this proposed mechanism: (i) the prevention of serine phosphorylation and IRS-1 degradation using a pharmacological inhibitor (rapamycin) was not associated with an improved acute response to insulin, (ii) protection against oxidative-stress insulin resistance by lipoic acid (LA) did not prevent IRS serine phosphorylation and IRS1 degradation, (iii) oxidative stress decreased PKB phosphorylation, an effect that was prevented by LA (as was the decrease in glucose uptake, but not increased serine phosphorylation of IRS). The magnitude of the effect of oxidative stress on PKB was greater than its effect on IRS-1 degradation, suggesting a greater degree of impairment as the insulin signal is propagated downstream\textsuperscript{89}. These results support the idea that oxidative stress-induced insulin resistance may not be limited to alterations in IRS function or content, and may involve other downstream sites. Additional studies will be required using targeted approaches, such as siRNA and transgenic mice, and physiological inducers of oxidative stress such as hyperglycaemia, FFA, and hyperinsulinaemia (either alone or in combination) to evaluate this hypothesis more effectively.

**Antioxidants and insulin resistance: Clinical studies**

Studies in animal models of diabetes indicate that antioxidants, especially \(\alpha\)-lipoic acid (LA), improve insulin sensitivity\textsuperscript{90}. There are several available antioxidants that hold promise as new approaches for the treatment of insulin resistance, including \(N\)-acetyl cysteine, \(\alpha\)-lipoic acid (LA), and flavanols. A number of studies have found that the antioxidants LA, glutathione, vitamin E, and vitamin C increase insulin sensitivity in patients with insulin resistance, T2D, and/or cardiovascular disease.

In patients with T2D, both acute and chronic administration of LA improves insulin resistance as measured by both the euglycaemic-hyperinsulinaemic clamp and the Bergman minimal model\textsuperscript{91-96}. In addition, the short-term (6 wk) oral administration of a novel controlled release formulation of LA lowered plasma fructosamine levels in patients with type 2 diabetes\textsuperscript{97}.
\(\alpha\)-lipoic acid: LA is an eight-carbon fatty acid that functions naturally as a cofactor in several mitochondrial enzyme complexes responsible for oxidative glucose metabolism and cellular energy production\(^95\). LA has been prescribed in Germany as a pharmacological antioxidant for over 30 years for the treatment of diabetes-induced neuropathy, and this compound is safe, well tolerated and efficacious\(^98\). Interestingly, several clinical studies have reported an improvement in insulin sensitivity and whole-body glucose metabolism in patients with T2D after intravenous infusion of LA\(^95\). Oral administration of LA (enteric-coated tablet) exerts a smaller (~20%) but significant effect\(^95\). To overcome the abbreviated half-life of LA (~30 min), a controlled release, orally available formulation of LA (CRLA) has been developed, and significantly reduced plasma fructosamine in patients with T2D\(^97\).

Although the exact mechanism of action of LA is unknown, *in vitro* data have indicated that LA pretreatment maintains the intracellular level of reduced glutathione (the major intracellular antioxidant) in the presence of oxidative stress, and blocks the activation of serine kinases that are associated with insulin resistance\(^34,62,99,100\). Thus, LA may preserve the intracellular redox balance (acting either directly or through other endogenous antioxidants such as glutathione), thereby blocking the activation of inhibitory inflammatory serine kinases including IKK\(\beta\)\(^33\) (Fig. 2). Alternatively, LA might increase insulin sensitivity through its tissue-specific effects on AMP kinase (AMPK)\(^101\), which have recently been described\(^102,103\).

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)\(^36\). In healthy subjects, infusion of FFA (as Intralipid) causes increased oxidative stress as judged by increased malondialdehyde levels and a decline in the plasma reduced/oxidized glutathione ratio\(^36\). Malondialdehyde, a highly toxic byproduct generated in part by lipid oxidation and ROS, is increased in diabetes mellitus\(^104\). In both normal individuals and in subjects with T2D, restoration of redox balance by infusing glutathione improves insulin sensitivity along with \(\beta\)-cell function\(^105\).

N-acetylcysteine (NAC): N-acetylcysteine (NAC), a thiol-containing antioxidant that elevates intracellular glutathione levels\(^106\), is receiving growing attention for potential use as a therapeutic agent in clinical settings in which there is evidence of increased oxidative stress\(^107,109\). Polycystic ovary syndrome (PCOS) occurs in 5 to 10 per cent of women of reproductive age and is clinically characterized by the hyperandrogenism and anovulation\(^110,111\). Moreover, up to 40 per cent of PCOS patients, both lean and obese, are also insulin resistant and hyperinsulinaemic. These insulin resistant PCOS patients are at high risk of developing T2D and the metabolic syndrome. Information about oxidative stress in PCOS is beginning to emerge. In one report of 30 women with PCOS, a decrease in total antioxidant status was noted compared to controls\(^112\). In another study, an increase in free radical activity in the peritoneal fluid environment of infertile women, some of whom had PCOS, was noted\(^113\). In yet another study, an increase in oxidant and a decrease in antioxidant status were noted in 27 women with PCOS compared with 18 age- and body mass index-matched healthy women\(^114\). More recently, it has been reported that, compared with healthy women, those with PCOS had significantly elevated serum malondialdehyde, homocysteine, insulin resistance, and lipoprotein a levels, and significantly decreased serum total antioxidant status\(^115\). Although pharmacological agents such as
metformin or thiazolidinediones that improve insulin resistance, have been suggested as one approach\textsuperscript{115,116}, the long-term safety of these therapies has not been established in this patient group.

Data have been published indicating that NAC significantly increases (\(P<0.5\)) insulin sensitivity in women with PCOS\textsuperscript{117}. In addition to increasing insulin sensitivity, NAC significantly reduced the area under the curve for both insulin and C-peptide following an oral glucose tolerance test. These results provide support for the rationale of evaluating other antioxidants to improve insulin sensitivity in women with PCOS, with the ultimate goals of increasing ovulation, and reducing their risk for the development of T2D and cardiovascular disease.

\textbf{Vitamin C:} In addition to playing a major role in the aetiology of diabetic macroangiopathy, endothelial dysfunction could promote insulin resistance\textsuperscript{118}. It is possible that oxidative stress-mediated blunting of nitric oxide action indirectly affects insulin sensitivity (\textit{e.g.}, reduced peripheral blood flow, increased peroxynitrite formation, others) 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 (FMD) of brachial artery] were evaluated in smokers, non-smokers with impaired glucose tolerance, and non-smokers with normal glucose tolerance\textsuperscript{119}. Both insulin sensitivity and FMD were blunted in smokers and nonsmokers 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\textsuperscript{120}. In contrast, vitamin C had no effect in healthy controls.

In contrast to these promising results, a recent study concluded that high dose oral vitamin C therapy was ineffective at improving endothelial dysfunction and insulin resistance in T2D\textsuperscript{121}. Plasma vitamin C levels in 109 diabetic subjects (36 ± 2 \(\mu\)M) were lower than that observed in healthy individuals (>80 \(\mu\)M). Thirty two diabetic subjects with low plasma vitamin C (<40 \(\mu\)M) were subsequently enrolled in a randomized, double-blind, placebo-controlled study of vitamin C (800 mg/day for 4 wk). Insulin sensitivity (determined by glucose clamp) and forearm blood flow in response to acetylcholine (ACh), sodium nitroprusside (SNP), or insulin (determined by plethysmography) were assessed before and after 4 wk of treatment. In the placebo group (\(n=17\) subjects), plasma vitamin C (22 ± 3 \(\mu\)M), fasting glucose (159 ± 12 mg/dl), insulin (19 ± 7 \(\mu\)U/ml), insulin sensitivity (determined by euglycaemic clamp), and forearm blood flow did not change significantly after placebo treatment. In the vitamin C group (\(n=15\) subjects), basal plasma vitamin C (22 ± 3 \(\mu\)M) increased to 48 ± 6 \(\mu\)M (\(P<0.01\)) after treatment, but this was significantly less than that expected for healthy subjects. No significant changes in fasting glucose (159 ± 12 mg/dl), insulin (19 ± 7 \(\mu\)U/ml), insulin sensitivity, or forearm blood flow in response to ACh, SNP, or insulin were observed after vitamin C treatment. It is important to note that supplementation...
resulted in incomplete replenishment of vitamin C levels, and the statistical power of this study was limited due to the small sample size.

**Vitamin E:** Initial reports of a positive effect of vitamin E on insulin action in insulin resistant patients with type 2 diabetes were published over ten years ago. Twenty-five patients with type 2 diabetes were treated with vitamin E (d-\( \alpha \)-tocopherol; 900 mg/day) or placebo for three months in a double-blind, crossover design. There was a trend in the reduction of plasma glucose, along with significant reductions in HbA1c levels (7.8 vs. 7.1), triglycerides, free fatty acids, total cholesterol, low-density lipoprotein cholesterol, and apoprotein B. The \( \beta \)-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 euglycaemic-hyperinsulinaemic clamp.

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

Vitamin E also improved insulin action in elderly people. Twenty elderly, non obese subjects with normal glucose tolerance were submitted to euglycaemic-hyperinsulinaemic clamp in a double-blind, crossover, and randomized study after 4 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 4 wk, 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 euglycaemic-hyperinsulinaemic clamp. 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 reduced glycated haemoglobin, insulin, norepinephrine, and the homeostatic model assessment index, indicative of increased insulin sensitivity and improved glycaemic control.

More recently, the effect of vitamin E on endothelial function, insulin action and cardiovascular risk markers in young healthy adult offspring of parents with T2D was determined. Healthy, glucose-tolerant adults (18-38 yr), 14 (12 male/2 female) with at least one parent with T2D, and 14 (12 male/2 female) subjects with no family history of diabetes (controls) were studied. Insulin action was assessed by euglycaemic hyperinsulinaemic clamp (1 mU/kg/min). Endothelial function was assessed by forearm blood flow (FBF) responses to intra-brachial artery infusions of ACh (endothelium-dependent vasodilation), sodium nitroprusside (SNP) (endothelium-independent vasodilation) and N(G)-monomethyl L-arginine (LNMMA) (nitric oxide synthase inhibition). Thirteen offspring (18-38 yr, 11 male/2 female, BMI < 30 kg/m²) completed a randomized, double-blind, crossover trial (12 wk vitamin E 800 IU/day or placebo, 6 wk washout).
It was reported that exogenous glucose infusion rates to maintain euglycaemia were positively associated with response to acetylcholine in offspring \((r = 0.61, P<0.05)\), and were linked with triglycerides. Vitamin E had no effect on endothelial function, insulin action or cardiovascular risk markers in healthy adult offspring of parents with Type 2 diabetes. The authors concluded that these results supported a positive association between insulin action and endothelial-dependent vasodilation in young healthy adult offspring of parents with T2D, but indicated no effect of vitamin E on these parameters.

It is important to note that these trials have been relatively small and of short duration. These antioxidants need to be further evaluated in larger, double-blind, randomized, placebo-controlled studies of longer duration. Ideally, these trials would include the measurement of multiple indices of oxidative stress, plasma levels of antioxidants, and measures of insulin sensitivity and glycaemic control. Nonetheless, there is some supporting evidence, albeit not entirely consistent, indicating the beneficial effects on insulin action reported following treatment with the antioxidants LA, vitamin E, and vitamin C. These results also 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.

**Flavanols:** There is a growing body of epidemiological evidence supporting the idea that diets rich in fruits and vegetables reduce or delay the onset of many chronic diseases, including cardiovascular and related metabolic diseases\(^{129}\). A major class of compounds present in fruits and vegetables that is associated with cardioprotective effects is the flavanols\(^{130}\). Cocoa is also rich source of flavanols, a class of polyphenolic antioxidant compounds [e.g., (-)-epicatechin, (+)-catechin, procyanidins] found in plants\(^{130}\). In addition to their antioxidant activity, flavanols have also been associated with increased in nitric oxide bioavailability\(^{131,132}\). Consumption of chocolate can result in significant increases in plasma epicatechin concentrations and decreases in plasma baseline oxidation products\(^{133}\). Since nitric oxide bioavailability deeply influences insulin-stimulated glucose uptake and vascular tone, flavanols have been evaluated for potential positive metabolic and pressor effects. Flavanoid-rich dark chocolate (100 g) improved endothelial function and was associated with an increase in plasma epicatechin concentrations in healthy adults\(^{133,134}\).

The effects of either dark or white chocolate bars on blood pressure and glucose and insulin responses to an oral glucose tolerance test in healthy subjects was compared\(^{135}\). After a 7-day cocoa-free run-in phase, 15 healthy subjects were randomly assigned to receive for 15 days either 100 g dark chocolate bars, which contained approximately 500 mg polyphenols, or 90 g white chocolate bars, which contained no polyphenols. Successively, subjects entered a further cocoa-free washout phase of 7 days, and then were crossed over to the other condition. Oral glucose tolerance tests were performed at the end of each period to calculate the homeostasis model assessment of insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI); blood pressure was measured daily. HOMA-IR was significantly lower after dark than after white chocolate ingestion \((0.94 \pm 0.42\) compared with \(1.72 \pm 0.62; P<0.001)\), and QUICKI was significantly higher after dark than after white
chocolate ingestion (0.398 ± 0.04 compared with 0.356 ± 0.02; P=0.001). Although within normal values, systolic blood pressure was lower after dark than after white chocolate ingestion (107.5 ± 8.6 compared with 113.9 ± 8.4 mm Hg; P<0.05). These results suggest that dark but not white chocolate decreases blood pressure and improves insulin sensitivity in healthy persons.

These same researchers have also evaluated the effects of dark chocolate (DC) consumption on blood pressure (BP), flow-mediated dilation (FMD), oral glucose tolerance (OGTT), and insulin sensitivity in patients with essential hypertension (EH)\(^{136}\). After a 7-day chocolate-free run-in phase, 20 never-treated, grade I patients with EH (10 males; 43.7 ± 7.8 yr) were randomized to receive either 100 g per day DC (containing 88 mg flavanols) or 90 g per day flavanol-free white chocolate (WC) in an isocaloric manner for 15 days. After a second 7-day chocolate-free period, patients were crossed over to the other treatment. Noninvasive 24 h ambulatory BP, FMD, OGTT, serum cholesterol, and markers of vascular inflammation were evaluated at the end of each treatment. The HOMA-IR, QUICKI, and insulin sensitivity index (ISI) were calculated from OGTT values. Ambulatory BP decreased after DC (24 h systolic BP -11.9 ± 7.7 mm Hg, P<0.0001; 24 h diastolic BP -8.5 ± 5.0 mm Hg, P<0.0001), but not WC. DC but not WC decreased HOMA-IR (P<0.0001), and it improved QUICKI, ISI, and FMD. DC also decreased serum LDL cholesterol (from 3.4 ± 0.5 to 3.0 ± 0.6 mmol/l; P<0.05). In summary, DC decreased BP and serum LDL cholesterol, improved FMD, and enhanced insulin sensitivity in patients with EH. Additional studies in larger groups and in individuals with T2D will be needed to confirm these results.

**Conclusions and implications**

The molecular mechanisms whereby oxidative stress causes insulin resistance are undefined. In a variety of tissues, hyperglycaemia 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-κB, 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 impaired 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.

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