Diabetes is a disease characterized by poor glycemic control for which risk of the type 2 form increases with age. A rise in blood glucose concentration causes increased oxidative stress which contributes to the development and progression of diabetes-associated complications. Studies have shown that primary antioxidants or genetic manipulation of antioxidant defenses can at least partially ameliorate this oxidative stress and consequentially, reduce severity of diabetic complications in animal models. Data from humans is less clear and will be summarized in this review. We highlight results from studies performed to investigate the role of vitamin E in preventing diabetes-induced oxidative damage in cell culture, animal models, and human participants, and summarize evidence testing whether this nutrient has an effect on outcomes related to the diabetic complications of nephropathy, retinopathy, and neuropathy. The most compelling evidence for an effect of vitamin E in diabetes is on protection against lipid peroxidation, whereas effects on protein and DNA oxidation are less pronounced. More studies are required to make definitive conclusions about the effect of vitamin E treatment on diabetes complications in human subjects.

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kidney, eye, and nervous system, for which the end results are often end-stage renal disease, blindness, and limb amputation, respectively. Because this damage occurs as a result of increased ROS production, extensive investigation has evaluated the ability of antioxidants to ameliorate complications of diabetes. Indeed, antioxidants like N-acetyl-cysteine (NAC) (Zheretitskaya et al., 2009), vitamin C (Lino et al., 2005), and α-lipoic acid (Lin et al., 2006) are effective in reducing complications, indicating that it may be beneficial for diabetics to either increase antioxidant intake through normal dietary means or through supplementation. It must be noted, however, that previous clinical trials using antioxidant therapy for diabetics have shown both promise and inconsistent results, at times due to limitations of the studies (reviewed in Johansen et al., 2005). The consideration for either monitoring intake of a particular antioxidant through diet or through supplementation must include careful analysis of data, notably the role of physiologically relevant antioxidant concentrations to optimally mitigate diabetic complications with minimum side effects.

The role of vitamin E as the body’s primary lipid-soluble antioxidant (Wang and Quinn, 1999) makes it a good candidate for investigation of effects against diseases of aging, especially those that involve ROS as a main component. Vitamin E is a lipid soluble group of compounds with similar biological activity to RRR-α-tocopherol. This includes the stereoisomers α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol, along with the 4 tocotrienols—α, β, γ, and δ-tocotrienol [Fig. 1]. While most studies on vitamin E are performed with α-tocopherol, it is important to note that the other tocopherol and tocotrienol stereoisomers and their metabolites appear to have potent biological properties. Many of these properties appear to be independent of the antioxidant functions of vitamin E. For example, α-tocopherol has been shown to influence cell signaling by inhibiting protein kinase C (PKC) (Azzi et al., 1997). Gene regulatory functions have also been reported for vitamin E as the tocophorons have been shown to potentially serve as ligands for the transcription factor peroxisome proliferator-activated receptor-γ (PPAR-γ) (De Pascalle et al., 2006). Evidence for these roles of the vitamin have been reviewed thoroughly by Traber and Atkinson (2007). Among their conclusions was that the primary role of vitamin E is as a lipid peroxyl radical scavenger in vivo and this role likely mediates the effects vitamin E has on cell signaling pathways. In light of this, we focus this review on the potential actions of vitamin E as an antioxidant and a potential regulator of endogenous antioxidant defenses in diabetes complications.

Because it is lipid soluble, ingested vitamin E is absorbed with lipids, packaged into chylomicrons, and transferred to the liver, after which it appears in plasma due to the expression of α-tocopherol transfer protein in the liver (Brigelius-Flohe and Traber, 1999). Once in tissue, vitamin E resides in the membranes of the cell, where it primarily serves as a chain-breaking antioxidant to prevent lipid peroxidation. With its potent antioxidant properties, high serum vitamin E concentrations have been associated with reduced risk of diseases like cardiovascular disease (Munteanu et al., 2004) and cancer (Knekt et al., 1988).

There is currently a strong interest in the effects of vitamin E and its relationship to diseases of aging. It is therefore logical to investigate associations between vitamin E and a disease like diabetes, particularly because of the multiple mechanisms by which ROS are generated by elevated blood glucose. Studies have shown that plasma α-tocopherol concentrations are lower in diabetics compared to controls (Nourooz-Zadeh et al., 1997), and appear to be even lower in diabetics with complications like microangiopathy than diabetes without complications (Martingallan et al., 2003). Epidemiological studies have examined the associations between vitamin E intake and diabetes outcomes. Serum α-tocopherol concentrations have been found to be an independent predictor of type 2 diabetes development in a seven year follow-up study in 50-year-old non-diabetic Swedish men (Arnlov et al., 2009). More specifically, the highest tertile of serum α-tocopherol had a 46% lower risk of development of type 2 diabetes than the lowest tertile, thus providing a relationship between physiological vitamin E status and risk of diabetes. Mayer-Davis et al. (2002) found that mean vitamin E intake is not associated with development of type 2 diabetes, but high plasma α-tocopherol may be protective. Additional studies have confirmed a relationship between low vitamin E status and increased risk of type 2 (Salonen et al., 1995) and type 1 diabetes (Knekt et al., 1999). This trend is not universal as some studies report no effect of good vitamin E status or use of supplements. Uusitalo et al. (2008) reported no significant association between high serum concentrations of α- or γ-tocopherol and advanced β-cell autoimmunity, as indicated by the presence of type 1 diabetes autoantibodies. Additionally, 600 IU vitamin E supplementation on alternate days showed no reduced risk against the development of type 2 diabetes over placebo at a 10-year follow-up (Liu et al., 2006). Notwithstanding these results, the majority of the data do support a possible link between vitamin E status and the risk of developing type 2 diabetes, which warrants further study of the concentrations most associated with type 2 diabetes risk.

This review will concentrate on the role of vitamin E as an antioxidant in diabetes complications with a focus on maintenance of cellular macromolecule components. Additionally, we will highlight how macromolecule maintenance appears to translate to protection of tissues most damaged by diabetes, including the pancreas, kidney, eye, and nervous tissue. We will also summarize evidence for an indirect antioxidant role of vitamin E and its metabolites in potential transcriptional regulation of antioxidant enzymes.

2. Protection against oxidative stress in diabetes by vitamin E

2.1. Intracellular concentrations of ROS

Previous work with fluorescent probes (Obrosova et al., 2005) and spin trapping agents (Gille et al., 2002; Stadler et al., 2008) has shown that diabetes increases ROS generation and that this rise in ROS concentrations can be prevented by antioxidants such as NAC or taurine (Tang et al., 2007). For example, glomeruli of diabetic
rats stained with 2,7'-dichlorofluorescin diacetate (DCFH-DA) displayed higher fluorescence in comparison to controls, indicating an increase in ROS production due to the diabetic state (Koya et al., 2003). This increase in fluorescence was attenuated by the treatment of diabetic rats with vitamin E or the antioxidant protocol, which is used primarily to inhibit LDL oxidation in atherosclerosis. When examining superoxide radical generation specifically, Rhee et al. (2005) found that superoxide radical concentrations in liver of streptozotocin-induced diabetic rats were significantly higher than that of control livers. Vitamin E supplementation reduced liver superoxide radical concentrations in a dose-dependent manner. In a rat model for type 2 diabetes, Minamiyama et al. found a significant rise in superoxide radical generation in cardiovascular tissue, which was reduced by α-tocopherol rather than not γ-tocopherol (Minamiyama et al., 2008). The reason for the ineffectiveness of γ-tocopherol is unclear as the authors cited studies demonstrating efficient antioxidant capabilities of γ-tocopherol (Christen et al., 1997). This discrepancy may be due to transport and localization of the separate tocopherol isomers rather than antioxidant mechanisms. When the antioxidant capabilities of vitamin E were examined clinically in healthy, overweight subjects, Manning et al. found that vitamin E supplementation lowered glucose concentrations, insulin levels, and peroxide concentrations in the plasma of participants at a 3-month time point (Manning et al., 2004). However, these effects were not observed at a 6-month time point, indicating no long-term effects of vitamin E supplementation on these outcomes. Most evidence is supportive of the notion that diabetes enhances the production of ROS and the subsequent elevated concentrations of ROS are lowered by vitamin E in experimental studies. It is unclear for the diabetic patient whether a long-term decrease in ROS concentrations can be achieved through maintenance of physiological vitamin E status by maintaining adequate intake from food or through supplementation.

2.2. Lipid peroxidation

Vitamin E is a powerful antioxidant that has been shown to decrease several outcomes of oxidative stress and oxidative damage in cell culture, in animal models of diabetes, and in diabetic humans. Because vitamin E is located in membranes and serves to reduce lipid peroxidation primarily as a chain-breaking antioxidant, most studies have examined the effects of vitamin E on lipid peroxidation products. This can be accomplished through the examination of TBARS (thiobarbituric acid reactive species) or by measuring specific products of lipid damage like malondialdehyde (MDA) or F2-isoprostanes. Outcome measures like these can be used to assess diabetes-induced lipid peroxidation in both experimental and clinical studies and to determine the extent by which vitamin E ameliorates effects on lipid damage. For example, Montero et al. (2000) observed that murine mesangial cells and rat glomerular endothelial cells cultured under high glucose conditions both had higher F2-isoprostane concentrations when compared to cells cultured under normal glucose concentrations. Furthermore, streptozotocin-induced diabetic rats placed on a high vitamin E diet (1000 IU/kg diet) exhibited significantly lower plasma and urinary F2-isoprostanes than diabetic controls on a normal diet. Besides affecting the concentration of lipid peroxidation products in plasma and urine, vitamin E given to diabetic animals has been shown to exhibit effects in several tissues as well, including kidney (Kuhad and Chopra, 2009), retina (Di Leo et al., 2003) and lens (Nazioglu et al., 1999), peripheral nerve (Nickander et al., 1994), brain (Tuzcu and Baydas, 2006), and liver (Rhee et al., 2005).

Data from clinical studies demonstrates a role for vitamin E to decrease lipid peroxidation in diabetic humans. Subjects with metabolic syndrome who were supplemented for 6 weeks with 800 mg/day α-tocopherol, γ-tocopherol, or both had lower blood MDA + 4-hydroxynonenal (HNE) and lipid peroxides than the subjects supplemented with placebo (Devaraj et al., 2008). Similarly, a study of type 1 diabetic children showed that 100 IU vitamin E/day lowered erythrocyte MDA and increased erythrocyte glutathione (Jain et al., 2000). Other studies have confirmed a similar effect through measurement of TBARS in blood and indicate that MDA in diabetics can be decreased by supplementation with vitamin E (Jain et al., 1996; Mol et al., 1997). Vitamin E supplementation also decreased in vitro erythrocyte (Manuel y Keenoy et al., 2001) and lipoprotein (Engelen et al., 2000) TBARS formation in middle-aged type 1 diabetics. For type 2 diabetics, 8 weeks of daily supplementation with 800 IU vitamin E combined with β-carotene and ascorbate decreased lipoprotein TBARS formation following in vitro oxidation (Anderson et al., 1999). The effects of vitamin E may not be sustained as Engelen et al. (2000) observed a return to baseline lipoprotein peroxidizability after vitamin E supplementation had ended, leading the authors to propose that diabetics should consider long-term or life-long supplementation in order to allow full benefit.

F2-isoprostanes can be affected by vitamin E in diabetic humans as well. Urinary F2-isoprostane levels have been found to be higher in both type 1 and type 2 diabetics when compared to controls and two weeks of vitamin E supplementation (600 mg/day) lowered urinary F2-isoprostanes for type 2 diabetics (Davi et al., 1999). This was confirmed when another study found a decrease in F2-isoprostanes after supplementation with 500 mg/day vitamin E for 6 weeks in type 2 diabetics (Ward et al., 2007; Wu et al., 2007). It should be noted for these studies that F2-isoprostanes were significantly lower in plasma due to vitamin E supplementation, but there was no difference in 24 hour urinary F2-isoprostanes. While urinary F2-isoprostanes have emerged as standard markers of lipid peroxidation, plasma F2-isoprostanes remain a strong validation tool (Kadiiska et al., 2005). Urinary F2-isoprostanes have several advantages in analysis (Montuschi et al., 2004), but there is still some debate regarding the diurnal and day-to-day variation in urinary F2-isoprostane concentrations in human subjects (Helmerson and Basu, 1999, 2001).

Reducing lipid peroxidation is particularly important not only as this indicates lower levels of oxidative stress but also because the biological products of lipid peroxidation can be quite reactive. MDA has the capability to interact with and bind to proteins, potentially rendering a vital protein to be nonfunctional. MDA can induce oxidative stress by targeting mitochondrial complexes I and II and thereby disrupting proper flow of electrons through the electron transport chain (Long et al., 2009). F2-isoprostanes can also pose a biological threat. Unlike MDA, which has a general mechanism of action by chemically modifying and influencing function of macromolecules, F2-isoprostanes appear to exert effects by receptor-mediated actions (reviewed in Comporti et al., 2008). In rat glomerular endothelial cells, F2-isoprostanes increased TGF-β, which itself has been implicated in the progression of diabetic nephropathy (Montero et al., 2000). The etiology of diabetes complications may be mediated in part by lipid peroxidation products, the formation of which may be inhibited by vitamin E. A decline in lipid peroxidation rate due to vitamin E treatment not only lowers oxidative stress and the resulting damage, but it may prevent deleterious biological effects caused by lipid peroxidation products.

2.3. Protein oxidation

In biological systems, structure determines function. This most aptly applies to proteins, of which particular residues are often oxidized under conditions of oxidative stress, altering structure
and often negatively affecting their function. For this reason, measuring the effect of any intervention on protein oxidation in diabetes not only provides insight into the level of oxidative damage but also provides an indirect measure of the efficiency and maintenance of protein components. Diabetes increases protein oxidation in diabetic humans (Telci et al., 2000) and in animal models of diabetes (Cumaoglu et al., 2007). Antioxidant interventions have shown to decrease oxidized protein levels in rats (Cumaoglu et al., 2007; Ardestani et al., 2008). Vitamin E reduces protein oxidation, which is often measured by quantification of protein carbonyl content by 2,4-dinitrophenylhydrazine (DNPH) derivatization and subsequent immunochemical detection. Vitamin E lowered protein carbonyl content in the livers (Je et al., 2001; Rhee et al., 2005) and brain mitochondria (Hong et al., 2004) of streptozotocin-induced diabetic rats. Vitamin E appears to lower protein carbonyl content in experimental diabetes, but its effect on protein carbonyls in human diabetics remains undocumented. It has been shown that young type 1 diabetics have higher plasma protein carbonyl content than controls and, within the diabetic group, plasma protein carbonyls were significantly higher in subjects with microangiopathy than subjects without this complication (Martin-Gallan et al., 2003). It is currently unclear how vitamin E might influence these trends. Vitamin E decreases protein carbonyl formation in experimental diabetes, but more work is required in order to conclude whether this trend is observed clinically.

Vitamin E may prevent glycation modifications to proteins that are likely to occur in diabetes. Vitamin E has been shown to inhibit the glycation of hemoglobin, which serves as a biomarker for the diagnosis of diabetes in a clinical setting, in both streptozotocin-induced diabetic rats (Je et al., 2001) and in a rat model for type 2 diabetes (Minamiyama et al., 2008). The mechanism by which vitamin E lowers protein glycation has been shown to be through inhibition of MDA formation, which contributes to the glycation of proteins in diabetics (Jain and Palmer, 1997). Furthermore, effects on protein glycation may be seen because vitamin E in vitro is a potent inhibitor of carboxymethyllysine formation from glycated human serum albumin, a reaction of glycated proteins that requires ROS in order to proceed (Schleicher et al., 1997). In humans, data examining the levels of glycated hemoglobin in diabetics following supplementation has provided mixed results. Vitamin E supplementation has been shown to decrease (Jain et al., 2000) or have no effect (Engelen et al., 2000) on the levels of glycated hemoglobin in type 1 diabetic subjects. Glycated hemoglobin and plasma protein concentrations were no different in type 1 and type 2 diabetic subjects who received eight weeks of α-tocopherol supplementation in comparison to a placebo group (Fuller et al., 1996). In another study using ten week vitamin E supplementation in type 2 diabetic subjects, there was no effect of treatment on any of several measures of protein oxidation or modification, including glycated albumin, glycated hemoglobin, glycated total plasma proteins, and glycated LDL (Reaven et al., 1995). Therefore, evidence does support an increase in both protein oxidation and glycation in diabetes and a suppressive effect of vitamin E on these parameters, but this data has come from studies using animal models. Currently, the human data that has examined an effect of vitamin E supplementation on damage is inconsistent. Efforts may be best spent in identifying any potential subsets of the diabetic population that respond most consistently to vitamin E supplementation in ameliorating protein modification in diabetes.

2.4. DNA damage

Oxidized DNA is a frequently employed marker of oxidative stress. Like the oxidation of lipids and proteins, the oxidation of DNA reveals information on the overall state of the system being investigated. DNA oxidation is of particular concern for mitotic tissues, where increases in DNA mutations are believed to increase risk for cancer development (reviewed in Halliwell, 2002). Data is most frequently collected by measurement of oxidized DNA bases in serum or excreted in urine, most notably 8-hydroxy-2′-deoxyguanosine (8-OH-dG), which has served as a biomarker for carcinogenesis (Valavanidis et al., 2009). Likely because of an overall higher rate of ROS generation than the general population, diabetic humans have a higher rate of DNA oxidation than controls as measured by serum 8-OH-dG (Pan et al., 2009). Epidemiological data has shown that diabetes is a risk factor for pancreatic (Batty et al., 2009) and ovarian cancer (Mori et al., 1998) but may not increase risk for endometrial cancer (Shoff and Newcomb, 1998). Diabetics have also been found to have higher risk of colorectal cancer, but diabetes does not appear to have an effect on short-term survival time (Jullumstro et al., 2009). The influence of diabetes on cancer risk depends on the population studied and the form of cancer as it has been demonstrated that diabetes is inversely related to prostate cancer risk (Kasper et al., 2009; Waters et al., 2009). However, data suggests that this is likely independent of oxidative stress and rather due to the effects of diabetes on hormone levels that influence the formation and progression of prostate cancer (Kasper et al., 2008). It is reasonable then that lowering the level of diabetes-induced DNA oxidation may reduce overall cancer risk. Finding a clear association between intake of particular antioxidants and reduced cancer risk is a difficult task (Halliwell, 2002). Data from long-term supplementation studies do not support a role for vitamin E in preventing prostate or total cancer in men (Gaziano et al., 2009) or in diabetic patients (Lonn et al., 2005), but a higher vitamin E status has been found to be associated with a lower risk for pancreatic cancer (Stolzenberg-Solomon et al., 2009). While the role of vitamin E in reducing cancer risk in diabetics is still unclear, lowering DNA oxidation in vivo may provide evidence that vitamin E reduces potential for mutation in tissues of diabetics. Insulin treatment in diabetic rats did not decrease kidney or liver 8-OH-dG unless administered with vitamin E, indicating a role for this vitamin in protection against DNA oxidation (Park et al., 2001). Clinically, it has been shown that DNA susceptibility to oxidation increases in diabetics, but that vitamin E does not affect DNA oxidation in either type 1 (Astley et al., 1999) or type 2 diabetics (Sampson et al., 2001). The limited amount of data to support a role for vitamin E in reducing DNA oxidation in animal models and humans requires more evaluation of the abilities that this and other antioxidants may have in maintaining the integrity of DNA in diabetes.

Vitamin E appears to maintain cellular components in experimental diabetes. Vitamin E suppresses diabetes-induced increases in ROS in both cell and animals models of diabetes complications. Diabetes models show increased concentrations of lipid peroxidation products, which vitamin E has been consistent in lowering. Literature results support an ability of vitamin E to lower lipid peroxidation in human diabetics as well. The study of protein modification has provided mixed results. Diabetes increases protein carboxylation and glycation and vitamin E lowers these outcomes in experimental models. However, clear trends have not emerged regarding an ability of vitamin E to perform these functions in humans. Finally, there is currently relatively little data to support a role for vitamin E in lowering DNA oxidation in both experimental diabetes and in human subjects.

3. Vitamin E influences on transcriptional regulation of antioxidant defense genes

There is evidence that vitamin E not only serves as a primary antioxidant but that it also influences the endogenous antioxidant
defense system. This system is composed of a series of antioxidant enzymes, detoxification enzymes, and transporters that are regulated by the transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Intracellular concentrations of Nrf2 are controlled by its repressor protein Keap1 by regulating the rate of Nrf2 degradation. Steady-state Nrf2 expression is typically quite low. However, under conditions of stress, the half-life of Nrf2 increases, as does the expression of enzymes and other proteins with genes containing upstream elements responsive to Nrf2, the so-called antioxidant response elements (Kobayashi and Yamamoto, 2006). Previously, Nrf2 has been shown to be protective in models of several diseases including Alzheimer's disease (Kanninen et al., 2008), Parkinson's disease (Chen et al., 2009), and chemically induced carcinogenesis (Pearson et al., 2008). Nrf2 has also been shown to be protective in models for diabetes. Xue et al. (2008) found that sulforaphane, a Nrf2-inducer, prevented an increase in ROS formation observed with human microvascular HMEC-1 endothelial cells that were incubated in high glucose media. Knockdown of Nrf2 under these conditions further increased ROS formation. Sulforaphane prevented high glucose-induced PKC activation and methylglyoxal accumulation. In a study of pancreatic β cells, upregulation of antioxidant enzymes by sulforaphane was found to prevent decreases in proliferation and viability due to cytokines IL-1β and IFN-γ (Song et al., 2009). Sulforaphane additionally prevented cytokine-induced production of H₂O₂ along with expression of COX-2 and iNOS and the resulting increases in PGE₂ and NO by inhibiting cytokine-induced NF-κB binding to DNA. Knockout of Nrf2 increases ROS formation and apoptosis in cardiomyocytes under high glucose conditions (He et al., 2009). Nrf2-knockout mice that were treated with streptozotocin had higher oxidative stress and diabetes-associated kidney damage than streptozotocin-treated controls (Yoh et al., 2008), suggesting a role for Nrf2 in delay of diabetic nephropathy. Insulin may act in part to protect human brain endothelial cells from high glucose stress by activating Nrf2 in a pathway that involves the insulin receptor tyrosine kinase, PI3K, Akt, and mTOR as upstream signaling proteins (Okouchi et al., 2006).

Because Nrf2 has been demonstrated to be protective in models of diabetes and its complications, then activation of this pathway by vitamin E would illustrate an indirect mechanism of protection against hyperglycemia-induced stress. There have been some efforts to elucidate potential effects that vitamin E and its metabolites have on Nrf2 activity or the endogenous antioxidant defense system as a whole. Pretreatment with vitamin E inhibits curcumin-induced heme-oxygenase-1 upregulation (HO-1) via Nrf2 in human hepatoma cells (Mcnally et al., 2007). This is likely because vitamin E lowers ROS concentrations, a certain level of which may be required to maintain an active Nrf2-response through oxidation of Keap1 cysteine residues, resulting in increased stability of Nrf2. However, if oxidative stress alone was vital to stimulate Nrf2 activity, then deficiency of vitamin E or selenium should upregulate Nrf2-responsive genes. It has been found that selenium deficiency, but not vitamin E deficiency, induces Nrf2 responses in mice (Burk et al., 2008). Therefore, there is little data to support a role for α-tocopherol or its deficiency in Nrf2-dependent antioxidant gene upregulation.

Other forms of vitamin E may participate in regulation of antioxidant defense mechanisms. α-Tocopherol-enriched mixed tocopherols induced Nrf2 and upregulated its downstream enzymes which resulted in suppressed prostate tumor development in mice (Barve et al., 2009). Additionally, the oxidized metabolite of γ-tocopherol, γ-tocopheryl quinone, increased GSH synthesis in an ATF4-dependent manner by enhancing cysteine availability (Ogawa et al., 2008). This effect was independent of Nrf2, which upregulates glutamate-cysteine ligase, the rate-limiting enzyme in GSH biosynthesis. Expectedly, γ-tocopheryl quinone had no effect on the expression of glutamate-cysteine ligase. In support of previous data, the equivalent α-tocopherol metabolite had no effect. Based on these observations, it appears that γ-tocopherol and not its metabolite γ-tocopheryl quinone may induce Nrf2 activity and consequent changes in gene expression while α-tocopherol, the predominant form used in supplementation studies, does not.

The influence of vitamin E on Nrf2 activity is highly dependent on the form of the vitamin administered, yet there is an abundance of data demonstrating that vitamin E – usually α-tocopherol – affects the expression of antioxidant enzymes. Consideration should be given to the possibility that α-tocopherol at least in part affects antioxidant enzyme expression through a more general mechanism like slowing the rate of protein and mRNA degradation due to oxidative stress. Vitamin E has been shown to influence the stability of mRNA. It has been previously reported that vitamin E increased glutathione peroxidase-1 (GPx1) activity, but not the activities of catalase or superoxide dismutase-1 (SOD1), and that this regulation was most likely due to increased mRNA stabilization rather than stimulation of transcription (Li et al., 1996). The mRNA levels and protein expression of the antioxidant enzyme heme-oxygenase-1 (HO-1) were increased in glomeruli of diabetic rats over controls (Koya et al., 2003). Treatment with vitamin E or the antioxidant BHA normalized this effect by lowering HO-1 mRNA and protein. It is likely then that these antioxidants did not directly decrease transcription of HO-1, but rather scavenged ROS to the extent that stress responses were not activated as occurred in untreated diabetic animals. HO-1 is primarily regulated by Nrf2, which the transcriptional activity can be increased in states of oxidative stress. Ameliorating oxidative stress may suppress the Nrf2 response. Opposite trends may have been observed more for enzymes like SOD1 and catalase because these have been found to be regulated by other transcription factors like PPAR-γ and NF-κB, the transcriptional activities of which are subject to influence by vitamin E (reviewed in Munteanu et al., 2004). Nakamura and Omaye demonstrated that vitamin E upregulates SOD1 and catalase and this may be mediated by PPAR-γ and NF-κB in human umbilical vein endothelial cells (Nakamura and Omaye, 2008). Hsieh et al. previously showed α-tocopherol to increase PPAR-γ mRNA (Hsieh et al., 2006), and both SOD1 and catalase have promoter region responsive elements for PPAR-γ and NF-κB. However, it has been previously reported that vitamin E inhibits NF-κB DNA-binding activity (Calfee-Mason et al., 2008). While it is very possible that vitamin E can affect PPAR-γ and NF-κB differently under diverse physiological conditions, more molecular work is currently required to decipher the exact mechanisms by which this essential nutrient influences the activity of PPAR-γ and NF-κB to increase expression of antioxidant genes. In all, different forms of vitamin E may stabilize mRNA of antioxidant genes, or activate transcription of the same genes via the regulatory factors Nrf2, PPAR-γ, and NF-κB.

4. Vitamin E influence on endogenous antioxidant defenses in diabetes

Maintenance of the antioxidant defense system is of particular interest in diabetes. In clinical studies, diabetic humans have been investigated with regard to activity and transcription of antioxidant defense enzymes. For example, three month vitamin E supplementation did not change enzyme activities for catalase, GPx, SOD1, or SOD2 in isolated human fibroblasts grown in either normal or high glucose conditions (Chiarelli et al., 2004). The results of a study of type 2 diabetic subjects did support a role for vitamin E in increasing activities of both GPx and SOD (Gokkusu et al., 2001). Similarly, Kutlu et al. (2005) reported that GPx, GSH, and vitamin E are all lower in the kidney and lens of diabetic rats.
when compared to non-diabetic controls and that these effects may be improved with exercise and supplementation with vitamins C and E. It has been observed that rats with streptozotocin-induced diabetes had lower SOD1 and catalase activity than control rats (Sindhu et al., 2004), but the activity of the GPx was found to be no different between groups. Treatment with insulin or antioxidants appeared to normalize these trends. Rat embryos cultured in diabetic culture media showed lower SOD1 and catalase activity in the brain, spinal cord, hearts, and liver compared to those cultured in control media (Zaken et al., 2001). SOD1 and catalase activity normalized following the addition of vitamins E and C into the diabetic culture media. It has been observed that the livers of diabetic rats on a vitamin E-supplemented diet had increased SOD1 activity over controls on a standard diet (Kinalski et al., 2000). Vitamin E increased liver GSH as well but had no effect on GPx activity. This effect may depend on the tissue being studied. Vitamin E administered alone increased GPx activity in red blood cells (RBCs) of diabetic rats and decreased GSH in the same cells (Naziroglu and Cay, 2001). When combined with selenium, vitamin E increased RBC GSH concentrations. Vitamin E had no effect on GPx activity in liver or muscle. In all, most evidence regarding an influence of vitamin E on endogenous antioxidant defenses points toward this nutrient normalizing the dysregulation caused by the diabetic state. Vitamin E may be acting as a scavenging antioxidant for lipid peroxyl radicals, thereby maintaining membrane integrity and reducing glucose-induced damage that might be responsible for dysregulation of antioxidant defenses.

Because vitamin E reduces the peroxidation of unsaturated fatty acids, its role would be of particular importance in tissue enriched in these fatty acids. The antioxidant capabilities of brain are of interest due to the enrichment of this tissue with omega-3 polyunsaturated fatty acids which tend to serve as susceptible targets of ROS attack. Diabetes decreased activity of SOD and GPx in the brain of rats (Hong et al., 2004). High-dose vitamin E supplementation (400 mg vitamin E/kg diet) was able to increase activity of both enzymes, but only SOD was found to recover to control non-diabetic activity levels.

There is little evidence that α-tocopherol directly induces the transcriptional upregulation of genes controlled by Nrf2. However, other forms of vitamin E like γ-tocopherol might contribute to Nrf2 activation. Vitamin E in general does appear to normalize the expression level of particular antioxidant enzymes under diabetic conditions. It is likely then that changes in antioxidant enzyme expression might occur as a result of oxidative stress and that vitamin E simply prevents some of these changes through its scavenging abilities.

5. Vitamin E protection against tissue damage in diabetes

5.1. The pancreas and glucose control

Central to the development and progression of both type 1 and type 2 diabetes are the pathophysiological changes that occur in the pancreas and in particular, the insulin-secreting β-cells. In type 1 diabetes, the β-cells undergo cell death primarily as a result of necrosis caused by an autoimmune response (reviewed in Robertson et al., 2003; Robertson and Harmon, 2006). The resulting depletion of functional β-cells leads to the type 1 diabetes phenotype. Although chemically induced, many animal studies have indicated a role for oxidative stress in development of streptozotocin-induced diabetes, as antioxidants have been found to slow or prevent pancreatic complications after administration of this agent (Takatori et al., 2004). Unlike type 1 diabetes, type 2 diabetes begins as a disease of insulin responsiveness, not insulin secretion. Therefore, type 2 diabetics often retain functional β-cells for some time after the onset of the disease (Robertson and Harmon, 2006). Over time and with progression of type 2 diabetes, capabilities for proper insulin secretion are often diminished. This appears to be because the disease state induces significant oxidative stress in the pancreas of rat models for type 2 diabetes (Ihara et al., 1999). The oxidative stress is accompanied by a substantial decrease in the number of insulin-secreting β-cells in the pancreas (Jin et al., 2008). The mechanisms by which oxidative stress gradually decreases insulin secretion over time appears to be through increased β-cell death and dysfunction in the transcriptional regulation of insulin via PDX-1 (pancreatic and duodenal homeobox 1) and Mafa (Tanaka et al., 2002; Harmon et al., 2005). Interestingly, low glucose also promotes oxidative stress and consequent apoptosis, a pathway suppressed by vitamin E (Cai et al., 2007). In diabetic rats, treatment with antioxidants lowers markers of oxidative damage in the pancreas (Jin et al., 2008). Additionally, antioxidant treatment was shown by immunohistochemical analysis to preserve the number of insulin-positive β-cells. Proper maintenance of antioxidant defenses might be effective for slowing progression of diabetes itself by sustaining functional pancreatic β-cells.

Oxidative stress is central to long-term β-cell dysfunction in type 2 diabetes and this effect can be mitigated by antioxidants. Treatment with antioxidants like α-lipoic acid has also been demonstrated to improve functional outcomes, like insulin sensitivity in type 2 diabetic subjects (Jacob et al., 1999). As an antioxidant, vitamin E improves outcomes related to pancreas physiology in diabetes (Sena et al., 2008), which may improve functional outcomes of diabetes in animal models. Asayama et al. (1986) found that rats deficient in vitamin E, selenium, or both had decreased insulin secretory reserves, suggesting that vitamin E status can directly affect pancreatic islet function. In a mouse model of type 2 diabetes, treatment with vitamin E combined with vitamin C and NAC resulted in larger and more numerous pancreatic islets than controls at 10 and 16 weeks (Kaneto et al., 1999). β-Cell number was increased at 16 weeks for the antioxidant combination treatment, which was found to be due to a decrease in β-cell apoptosis with no effect on proliferation. The antioxidant combination treatment maintained the PDX-1 expression at 16 weeks that was decreased in the diabetic state. The treatment of vitamin E + vitamin C + NAC preserved pancreatic islet morphology by decreasing β-cell apoptosis and functioning by maintaining PDX-1 expression in diabetic animals. However, further experimentation indicated that vitamin E had a minimal role in comparison to that of NAC alone. Vitamin E may play a role in pancreatic protection, but this role may be to act in concert with other antioxidants to exert protective effects.

Studies examining the effect of vitamin E supplementation on glucose control may provide additional yet indirect evidence that the nutrient maintains the pancreatic islet in diabetes. Supplementation with tocoferol for 10 weeks helped preserve body weight and lower plasma glucose concentrations with no effect on plasma insulin concentrations in streptozotocin-induced diabetic rats (Kuhad et al., 2009). Supplementation with vitamin E appeared to lower plasma glucose in type 2 diabetic humans (Paolisso et al., 1993; Gokkusu et al., 2001). However, Paolisso et al. (1993) concluded from their study with type 2 diabetics that 900 mg vitamin E/day lowers plasma glucose but may not improve pancreatic response to glucose. A question regarding human studies is how long the effects of supplementation might improve metabolic outcomes, if at all. Manning et al. (2004) reported that the effects of vitamin E may be transient as fasting plasma glucose and insulin concentrations were reduced after 3 months of supplementation, but these effects were lost when subjects were evaluated again at 6 months. While it may initially appear that vitamin E caused only a short-term benefit, it is important to note...
that the subjects were supplemented with 800 mg/day vitamin E for the first 3 months of the study and then increased to 1200 mg/day vitamin E for the second 3-month interval. Because all antioxidants can become prooxidants under particular conditions and at certain concentrations, it is very possible that the effects on metabolic outcomes were lost between months 3 and 6 because the 50% increase in daily vitamin E dose began to cause a detrimental effect, negating the improvements observed at 3 months. Additionally, the population used in the Manning study was one of overweight individuals with no diagnosis of diabetes or pre-diabetes. It would be interesting to see if the trends observed with regard to benefits of vitamin E on metabolic outcomes are specific to non-diabetic overweight individuals or if these results would be upheld in a diabetic population. Animal studies show a modest effect of vitamin E supplementation on glucose control in type 2 diabetes, suggesting that vitamin E would be best utilized when used in conjunction with other antioxidants. It is currently unclear as to the effect on insulin secretion in humans and the duration of the effect.

5.2. Diabetic nephropathy

It is well established that diabetes not only targets the pancreas, but also causes damage in several other tissues and organs, including the kidney, eye, peripheral nerves, and brain. Finding methods to alleviate some of the damage at these sites may reduce medical costs and significantly increase quality of life in diabetics. With regard to kidney disease, the rise in type 2 diabetes in recent decades has led to it now be the leading cause of end-stage renal failure (Harvey, 2003). There is data to support a preventive role for antioxidants like α-lipoic acid (Obrosova et al., 2003) and vitamin C (Iino et al., 2005) in diabetic nephropathy. Vitamin E and its status have also been implicated. Using regression analysis, plasma vitamin E served as an independent predictor of plasma creatinine in type 2 diabetic humans (Zitouni et al., 2005). Animal studies have shown vitamin E supplementation to reduce oxidative stress in glomeruli of diabetic rats (Koya et al., 2003). Oxidative stress in diabetic kidney is usually associated with tissue damage that interferes with proper organ function, causing an increase in urinary protein excretion and blood urea nitrogen (BUN) (Montero et al., 2000). Vitamin E supplementation (1000 IU/kg diet) for 4 weeks after streptozotocin-induction of diabetes resulted in significant reductions in both measures compared to diabetic rats on a control diet. Other data has confirmed an effect of vitamin E in reducing BUN and serum creatinine in diabetic rats, demonstrating a positive effect on kidney function (Haidara et al., 2009). At the cellular level, one mechanism of action has been proposed to be the inhibition of oxidative stress–induced NF-κB activation and apoptosis in rat kidney (Kuhad and Chopra, 2009). This data indicates that kidney function of diabetic animals can be improved by vitamin E. In a human trial involving supplementation with vitamins E and C together, those given the antioxidants had a significantly lower concentration of urinary microalbumin excretion compared to the placebo group (Farvid et al., 2005). This is of importance because elevated microalbumin in urine is indicative of kidney disease, especially in diabetics. More data is required to properly evaluate the ability of vitamin E to protect against kidney damage in diabetic patients, but if successful, antioxidant treatment may have a significant impact.

5.3. Damage to the eye

The eyes of diabetics are significantly affected by the disease. Diabetic retinopathy is the most common complication of the disease and threatens the sight of many people with diabetes (Negi and Vernon, 2003). Diabetic retinopathy has the signature characteristics of microvascular damage, haemorrhage, and lipid accumulation affecting the retina that may cause deterioration of a patient’s vision (Negi and Vernon, 2003). Previous interventions with antioxidants have shown benefit in retinal protection in diabetes (reviewed in Kowluru and Chan, 2007). Use of vitamin E as an intervention to prevent or slow outcomes of retinal degeneration requires examination of symptoms like the formation of acellular capillaries (capillaries lacking pericytes and endothelial cells) and pericyte ghosts (capillaries lacking pericytes but with one or more endothelial cells). When combined with vitamin C, vitamin E inhibited formation of acellular capillaries in diabetic rats (Kowluru et al., 2001). A statistically significant decrease in pericyte ghosts was not achieved with vitamin C and vitamin E together, but the impact was significant for the diabetic rats fed a diet containing vitamins C and E in combination with other antioxidants like Trolox, NAC, β-carotene, and selenium. Diabetic rats fed a diet with a mix of antioxidants, including vitamin E, showed an inhibition of diabetes–induced NF-κB activation, which is an early marker of diabetic retinopathy and is sustained through the development of the disease (Kowluru et al., 2003). Vitamin E also protected retinal endothelial cells against high glucose-driven caspase-3 dependent apoptosis (Kowluru and Koppolu, 2002). Clearly, vitamin E shows protective effects on the diabetic retina in animal and cell studies.

When humans have been studied, there was no relationship observed between vitamin E intake and severity of retinopathy in type 2 diabetes (Mayer-Davis et al., 1998; Millen et al., 2004). Millen et al. found there to be no association between risk of retinopathy and intake of vitamins C or E from foods and supplements (Millen et al., 2004). Therefore, vitamin E status from food intake or supplementation at the levels observed in these studies may not be sufficient to protect the retinas of diabetics against the severity of oxidative stress caused by the disease.

Diabetes not only causes retinal damage, but it also causes damage to the lens and conjunctiva, cornea, pupil, and optic nerve and may increase risk of glaucoma (Negi and Vernon, 2003). In addition, diabetes induces damage to the lens of the eye promoting the formation of cataracts, a clouding of the lens usually as a result of protein denaturation. In diabetics, this is due to oxidative modification of lens proteins which puts diabetics at a significantly higher risk of forming cataracts than non-diabetics (Klein et al., 1985). Animal studies have demonstrated that vitamin E plays a part in reducing lipid peroxidation (Kutlu et al., 2005) and thereby protecting and maintaining the lens of the diabetic eye (Naziroglu et al., 1999). In all, animal studies have shown some benefit of vitamin E on outcomes of eye health, but there is insufficient evidence for an impact at physiological levels of the vitamin to affect eye health in diabetic humans.

5.4. Diabetic neuropathy: the peripheral and central nervous systems

Diabetes has profound effects on the nervous system, including both peripheral nerves and the central nervous system. In the peripheral nervous system, diabetes causes a progressive deterioration of primarily sensory nerves, although motor nerves are observed to be damaged as well. Approximately 50% of diabetics experience some degree of neuropathy, which is ultimately the leading cause of lower extremity amputation (Obrosova, 2008). Peripheral neuropathy is thought to develop because of cellular damage to endothelial cells, affecting nerve blood flow, and to the neurons themselves, affecting conductivity of impulses (Obrosova, 2008). There is data to demonstrate that neurons suffer oxidative damage and undergo apoptosis in diabetes (Vincent et al., 2005). Results from our lab have confirmed this as the cell viability of the neuroblastoma PC12 cells decreased significantly under high
Antioxidants improve cell viability in PC12 cells cultured in high glucose. PC12 cells were cultured in high glucose (75 mM) ± 1 mM NAC for 72 h. An MTT assay of cell viability was performed and absorbances standardized as percent of normal glucose (25 mM) controls. Data is expressed as mean ± SE. Statistical difference of \( p < 0.0001 \) between treatment with and without NAC is denoted by an asterisk. (Fig. 2)

NAC treatment increases intracellular GSH concentrations in PC12 cells. PC12 cells were cultured in high glucose and were then scraped into PBS and analyzed for total GSH by HPLC with electrochemical detection. GSH concentrations were standardized to total protein. Data is expressed as mean ± SE. A statistical difference of \( p < 0.0001 \) between treatment with and without NAC is denoted by an asterisk. (Fig. 3)

**6. Conclusions**

It has been demonstrated that diabetes induces oxidative stress and the resulting damage may be mitigated by treatment with some antioxidants. However, each antioxidant exerts effects based

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**Fig. 2.** Antioxidants improve cell viability in PC12 cells cultured in high glucose. PC12 cells were cultured in high glucose (75 mM) ± 1 mM NAC for 72 h. An MTT assay of cell viability was performed and absorbances standardized as percent of normal glucose (25 mM) controls. Data is expressed as mean ± SE. Statistical difference of \( p < 0.0001 \) is denoted by an asterisk.

**Fig. 3.** NAC treatment increases intracellular GSH concentrations in PC12 cells. PC12 cells were cultured in high glucose and were then scraped into PBS and analyzed for total GSH by HPLC with electrochemical detection. GSH concentrations were standardized to total protein. Data is expressed as mean ± SE. A statistical difference of \( p < 0.0001 \) between treatment with and without NAC is denoted by an asterisk.
primarily on its mechanism of action. Mostly residing in cell membranes and lipoprotein particles, vitamin E appears to protect against macromolecule damage – especially lipid peroxidation – in experimental diabetes. There is also evidence to support a role of vitamin E in protection of the pancreas, kidney, eyes and nerves. Optimizing vitamin E status achieved through food intake or supplementation at recommended amounts may slow the progression of the tissue damage in diabetes, but more studies are needed before definitive conclusions can be drawn.

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