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Dietary Supplementation With Vitamin E Ameliorates Cardiac Failure in Type I Diabetic Cardiomyopathy by Suppressing Myocardial Generation of 8-*iso*-Prostaglandin F_{2α} and Oxidized Glutathione

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Abstract

Background—Diabetic cardiomyopathy has been documented as an underlying etiology of heart failure (HF) in diabetic patients. Although oxidative stress has been implicated in diabetic cardiomyopathy, much of the current evidence lacks specificity. Furthermore, studies investigating antioxidant protection with vitamin E in this unique cardiac phenomenon have yet to be performed. In the present study, we sought to determine whether vitamin E supplementation can confer cardioprotective effects against diabetic cardiomyopathy in relation to specific and quantitative markers of myocardial oxidative stress.

Methods and Results—Diabetes was induced in rats by a single injection of streptozotocin (STZ). Animals were fed either a basal diet or a diet enriched with 2000 IU of vitamin E/kg beginning immediately after induction of diabetes and continued for 8 weeks. Rats were examined for diabetic cardiomyopathy by left ventricular (LV) hemodynamic analysis. Myocardial oxidative stress was assessed by measuring the formation of 8-*iso*-prostaglandin F_{2α} (8-*iso* PGF_{2α}) as well as oxidized glutathione (GSSG). In the un-supplemented STZ-diabetic rats, LV systolic pressure (LVSP), rate of pressure rise (+dP/dt), and rate of pressure decay (−dP/dt) were depressed while LV end-diastolic pressure (LVEDP) was increased, indicating reduced LV contractility and slowing of LV relaxation. These hemodynamic alterations were accompanied by increased myocardial formation of 8-*iso* PGF_{2α} and GSSG. Vitamin E supplementation improved LV function and significantly attenuated myocardial 8-*iso* PGF_{2α} and GSSG accumulation in STZ-diabetic rats.

Conclusions—These findings demonstrate the usefulness of vitamin E supplementation during the early phases of type I diabetes for the prophylaxis of cardiomyopathy and subsequent HF.

Keywords

cardiomyopathy; oxidative stress; type I diabetes mellitus; heart failure; vitamin E

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Introduction

The majority of U.S. hospitalizations for complications of diabetes are linked to cardiovascular disease (1). Heart failure (HF) is a common and serious comorbidity of diabetes (1,2). Although the high incidence and poor prognosis of HF in diabetic patients is often explained by the coexistence of hypertension and/or myocardial ischemia, the absence of significant coronary obstructions in a subset of patients with diabetic HF strongly support the existence of an underlying diabetic cardiomyopathy (3–7). Diabetic cardiomyopathy has been found to be associated with depressed mechanical function which results in subclinical systolic and diastolic dysfunction (8,9). This diabetic cardiomyopathy has been documented to progress to congestive heart failure (CHF) in both type 1 and 2 diabetic patients. However, approximately 30% of all type 1 diabetic patients suffer from this specific cardiomyopathy (10,11). Despite these observations, no widely applicable treatment regimen has been developed for the prevention of cardiac complications of diabetes.

Available evidence from experimental models as well as observational studies have reported that diabetes increases oxidative stress and that the onset of diabetes and its complications, including diabetic cardiomyopathy, are closely associated with oxidative stress (12–17). In most of these studies, increased levels of thiobarbituric acid-reactive substances (TBARS) and lipid hydroperoxides have been used to establish an association between oxidative stress and diabetic complications. However, the use of these two classic markers of free-radical mediated oxidative stress has been documented to be problematic (18,19). Consequently, no definitive information as to the importance of this process *in vivo* in contributing to the functional alterations demonstrated in the hearts of diabetic patients as well as those of experimental animals is available.

Vitamin E has been shown to be a potent antioxidant and its presence in biological membranes is thought to represent the major defense system against free-radical mediated lipid peroxidation. Epidemiological studies have shown an inverse correlation between vitamin E intake and cardiovascular disease (20,21). However, interventional studies in patients have yielded conflicting results, with evidence both for (22–24) and against (25–29) the clinical value of vitamin E. It has recently been suggested that the incongruous results of vitamin E treatment in preventing cardiovascular complications may be due to the lack of identification criteria of patients who are most likely to benefit from antioxidant therapy (30) as well as inappropriate patient selection (31). No clinical trials to date have been performed in which patient enrollment was based on biochemical evidence of elevated oxidative stress. In that regard, persons with diabetes may be more prone to oxidative stress because hyperglycemia depletes natural antioxidants and facilitates the production of free radicals (16). Unfortunately, the vast majority of clinical studies have not been designed to assess the efficacy of vitamin E use specifically in diabetic patients. Thus, it is possible that vitamin E may be more efficacious in subpopulations who exhibit evidence of elevated oxidative stress. Given the number of shortcomings in the clinical trials, the potential of vitamin E to provide protection from myocardial dysfunction in situations of oxidative challenge remains open for further investigation.

Accordingly, the present investigation was designed to address these issues. F₂-isoprostanes are members of a newly described family of prostaglandin isomers that are produced *in vivo* primarily from oxidative modification of polyunsaturated fatty acids via a free radical-catalyzed mechanism (32). Of these, 8-*iso*-prostaglandin F_{2α} (8-*iso* PGF_{2α}) has recently been shown to be a specific and sensitive quantitative index of oxidative stress *in vivo* (33). Therefore, to evaluate myocardial oxidative stress in diabetic cardiac complications, we measured myocardial levels of 8-*iso* PGF_{2α} along with myocardial oxidized (GSSG) glutathione in relation to left ventricular (LV) hemodynamic function during type I diabetic

cardiomyopathy using the well-characterized rat streptozotocin (STZ) model of type I diabetes. To determine whether vitamin E is capable of conferring cardioprotective effects against diabetic cardiovascular disease, we supplemented STZ-diabetic rats with vitamin E. The results demonstrate that supplemental vitamin E provides significant protection against cardiac dysfunction and concomitant myocardial oxidative stress induced by type I diabetes. These findings provide evidence of a causal link between myocardial oxidative stress and diabetic cardiac dysfunction and support the use of vitamin E supplements for cardiovascular prevention of type I diabetic complications, especially cardiomyopathy.

Methods

Experimental Animals

Sprague-Dawley rats weighing 150 ± 10 g were obtained from Harlan (Indianapolis, IN). All experiments were performed in accordance with the protocols approved by the Institutional Animal Care and Use Committee at Meharry Medical College and conforms with the *Guide for the Care and Use of Laboratory Animals* of the US National Institutes of Health.

Induction of Diabetes

Hyperglycemia was induced in male Sprague-Dawley rats by administering a single intraperitoneal (i.p.) injection of streptozotocin (STZ) (65 mg/kg body wt) prepared daily in citrate buffer pH 4.5 for maximal stability. The control group was injected with the vehicle only. To ensure that the animals were diabetic (D), urine analysis was performed 24–48 hours after STZ injection by Chemstrip uGK (Roche Diagnostics, Indianapolis, IN). Rats with urine glucose values of >2000 mg/dL with polyuria 24–48 h after STZ injection were considered to be diabetic. Rats with urine glucose values of <2000 mg/dL 24–48 hours after STZ injection were not considered to be diabetic and were excluded from further study. After hyperglycemia was confirmed 24–48 h after STZ injection with a urine glucose test, the tests were then repeated weekly. All of the rats that were diabetic 24–48 h after STZ injection remained in a diabetic state through to the 8-week endpoint of our study as evidenced by continued urine glucose values of >2000 mg/dL along with polyuria.

Vitamin E Supplementation

Control and diabetic rats were randomly assigned to a standard rat chow [Rodent Diet (W) 8640] or a chow supplemented with vitamin E (2000 IU of tocopherol acetate/kg of feed) obtained from Teklad (Madison, WI) beginning immediately after confirmation of STZ-induced diabetes. Rats were kept on their respective diets for 8 weeks post-STZ injection so as to allow an adequate period of time for any beneficial effects to be seen. Body weight and average daily food consumption of all groups of rats were measured weekly. Animals were used at eight weeks after vehicle or STZ injection for different studies. Urine glucose values at 8 weeks in the STZ-diabetic rats receiving vitamin E were similar to that observed in the un-supplemented STZ-diabetic rats, indicating that the severity of diabetes was the same between the two groups.

Cardiac Function

Rats were anesthetized with ketamine-xylazine (90 mg/kg:10mg/kg i.p.). The right carotid artery was cannulated with a Millar miniature catheter (SPC-320, 2F, Millar Instruments, Houston, Texas) and advanced into the aorta to record arterial pressure. The aortic catheter was then advanced into the left ventricle (LV) for recording of the following pressures: left ventricular end-diastolic pressure (LVEDP); left ventricular systolic pressure (LVSP); rate of pressure rise (+dP/dt); and rate of pressure decay (−dP/dt). Heart rate (HR) and mean arterial

pressure (MAP) was also measured. After these assessments, the rats were killed, and the hearts were removed for further studies.

Measurement of Myocardial Oxidative Stress

8-iso PGF_{2α}—LV tissue was homogenized in 0.1 M phosphate, pH 7.4, containing 1 mM EDTA. An equal volume of 15% wt/vol KOH was added to the tissue homogenate and incubated for 60 minutes at 4°C. 2–4 volumes of ethanol containing 0.01% BHT was added to the sample and incubated for 5 minutes at 4°C and then centrifuged at 1,500 × g for 10 minutes to remove precipitated proteins. The supernatant was then decanted into a clean test tube and the ethanol was evaporated to < 10% v/v under a gentle stream of nitrogen. The samples were then reconstituted with EIA buffer, vortexed, and then analyzed for 8-isoprostane using a commercially available kit (8-Isoprostane EIA Kit, Cayman Chemical Company, Ann Arbor, MI) and expressed as picomoles/g tissue.

Reduced and Oxidized Glutathione

LV tissue was homogenized in 5–10 ml of cold buffer (i.e., 50 mM MES, pH 6–7, containing 1 mM EDTA) per gram tissue and centrifuged at 10,000 × g for 15 minutes at 4°C. Reduced (GSH) and oxidized (GSSG) glutathione levels was examined in the supernatants using a commercially available kit (Glutathione Assay Kit, Cayman Chemical Company, Ann Arbor, MI). Exclusive measurement of GSSG was accomplished by derivatizing GSH with 2-vinylpyridine. Values were expressed as μM/mg tissue.

Protein Determination and Statistical Analysis

Protein concentration of LV tissue samples were determined using the Modified Lowry Protein Assay Reagent Kit (Pierce Biotechnology). Data are expressed as the mean ± SEM. Group means were compared by one-way analysis of variance (ANOVA), and ANOVA followed by Bonferroni's test was used to identify differences between multiple groups. Comparisons between two groups was performed by Student's *t*-test. Values of *P* < 0.05 were considered significant. Statistical analysis was done using SigmaStat 3.5 software.

Results

General Observations

STZ-diabetic animals supplemented with and without vitamin E showed a significant decrease in body weight and heart weight compared with controls (Table 1). The results in Table 1 also show an increase in heart/body weight ratio in the STZ-diabetic animals maintained on a basal or vitamin E-supplemented diet compared to respective control animals. The development of diabetes was confirmed by a marked elevation in the urine glucose level (>2000 mg/dl). We chose not to measure blood glucose values as anesthetization is required and this process has been shown to be associated with an increased risk of mortality, particularly in rats that are chronically and severely diabetic, as is the case in the present study.

Food Consumption

STZ-diabetic animals supplemented with and without dietary vitamin E displayed an increase in food intake compared to respective control groups of animals at 1-week and 2-weeks post-STZ injection (Figure 1). However, the hyperphagia observed among the vitamin-E treated and untreated STZ-diabetic rats disappeared after week 2 (Figure 1). As shown in Figure 1, there was no significant difference observed in food intake between the vitamin E-supplemented and un-supplemented STZ-diabetic groups of animals at any time point throughout the study.

Ventricular Function

LV hemodynamic parameters were measured in vitamin E-supplemented and un-supplemented control and diabetic groups of rats for assessment of ventricular performance. Figure 2 shows LV pressure and LV dP/dt recordings obtained from control and diabetic rats supplemented with and without vitamin E. The un-supplemented diabetic rat displays a lower LVSP and a higher LVEDP compared to the control rat (Figure 2C). In addition, compared to the control rat, the un-supplemented diabetic rat also displays a depressed LV dP/dt (Figure 2C). The diabetic rat supplemented with vitamin E displays an increased LVSP and a reduced LVEDP concomitant with a higher LV dP/dt (Figure 2D).

Hemodynamic data are summarized in Table 2. Supplementation of diabetic rats with dietary vitamin E resulted in an improved LVSP, LVEDP, +dP/dt, and MAP as compared to the un-supplemented diabetic rats. The diabetic animals supplemented with dietary vitamin E also showed an increase in -dP/dt as compared to the un-supplemented diabetic group, however, the increase was not statistically significant (Table 2). HR was significantly decreased in both the vitamin E-supplemented and un-supplemented diabetic animals relative to their respective controls (Table 2). These data indicate that vitamin E improves LV systolic and diastolic function in diabetic hearts independent of HR.

Myocardial Oxidative Stress

The amount of free-radical mediated oxidative stress was determined by evaluation of myocardial levels of 8-*iso* PGF_{2α}; these data are shown in Figure 3. Myocardial levels of 8-*iso* PGF_{2α} in un-supplemented diabetic animals increased by 143% relative to respective control values (Figure 3). Supplementation of diabetic animals with vitamin E decreased myocardial 8-*iso* PGF_{2α} levels by 38% as compared to the un-supplemented diabetic group, indicating lower myocardial oxidative stress among the vitamin E-supplemented diabetic animals (Figure 3). However, myocardial 8-*iso* PGF_{2α} levels remained higher among the diabetic animals supplemented with vitamin E as compared to respective control animals (Figure 3).

Myocardial oxidized (GSSG) glutathione levels were also examined in addition to 8-*iso* PGF_{2α} to assess oxidative stress. Myocardial GSSG levels in the un-supplemented diabetic animals were significantly increased relative to respective controls (Figure 4). Supplementation of diabetic animals with vitamin E completely normalized myocardial levels of GSSG such that no difference in myocardial GSSG levels was observed between the vitamin E-supplemented diabetic animals and respective control animals (Figure 4). These data provide further evidence of reduced myocardial oxidative stress among the diabetic animals supplemented with vitamin E.

Discussion

The excess in-hospital mortality of diabetic patients results primarily from an increased incidence of HF (34). The higher incidence of pump failure among diabetic patients has been attributed in part to an underlying subclinical diabetic cardiomyopathy that is unrelated to large-vessel atherosclerosis (4,34,35). This unique form of non-atherogenic cardiomyopathy occurs in roughly 30% of all type 1 diabetic patients (10,11) due to the protracted course of abnormal glucose homeostasis. Although diabetic cardiomyopathy has been reported to be associated with an increase in oxidative stress (12–15), little information is available on the *in vivo* formation of F₂-isoprostanes in diabetic myocardium with attendant cardiomyopathy. Furthermore, no information is available on the effects of vitamin E on myocardial F₂-isoprostane formation in diabetic cardiac complications. From a broader perspective, the

biological significance of oxidative stress in diabetes and its complications remains poorly understood.

The present study, performed using the STZ-diabetic rat model of type I non-atherogenic cardiomyopathy, provides significant new information that is relevant to diabetic cardiac complications. The salient results can be summarized as follows: (1) in STZ rats with type I diabetic cardiomyopathy, myocardial levels of 8-*iso* PGF_{2α} and GSSG are significantly increased, a reflection of increased oxidative stress; (2) supplementation of STZ-diabetic rats with 2000 IU of vitamin E/kg food provides significant protection against diabetes-induced cardiac dysfunction; (3) vitamin E blunts diabetes-induced amplification of myocardial 8-*iso* PGF_{2α} and GSSG formation; and (4) excess myocardial oxidative stress is causally related to the development of type I diabetic cardiomyopathy. Taken together, these data demonstrate a causal role for oxidative stress in mediating the pathogenesis of type I diabetic cardiomyopathy and support antioxidant intervention with vitamin E as a cardioprotective strategy in the setting of type I diabetes.

Our results strengthen the concept of oxidative stress of hyperglycemia as a potential mechanism for diabetic cardiac complications. In the present study, we found myocardial levels of 8-*iso* PGF_{2α} to be increased in the LV of un-supplemented diabetic rats 8 weeks after STZ injection. The increased formation of myocardial 8-*iso* PGF_{2α} among the un-supplemented STZ-diabetic animals was associated with significant depressions in LVSP, +dP/dt, and -dP/dt and concomitant increases in LVEDP. These hemodynamic alterations in the un-supplemented STZ-diabetic rats demonstrate abnormal LV systolic and diastolic function that is the hallmark of diabetic cardiomyopathy. Interventional experiments involving the antioxidant vitamin E attenuated these hemodynamic changes and significantly depressed myocardial 8-*iso* PGF_{2α} in the STZ-diabetic rats. To our knowledge, it has not previously been shown that vitamin E supplementation can significantly affect diabetes-induced accentuation of specific F₂-isoprostanes, such as 8-*iso* PGF_{2α}, in the hearts of STZ-diabetic rats. These findings provide strong evidence of a causative role for myocardial oxidative stress in the development of type I diabetic cardiomyopathy.

The significantly lower HR observed in untreated STZ-diabetic rats in the present study is in agreement with previous findings from the literature (36–40). It is known that reductions in HR are routinely associated with cardiac depression. However, studies undertaking functional comparison at comparable heart rates via pacing in STZ-diabetic rats have reported similar alterations in myocardial contractility despite correcting for HR (41,42). Therefore, the hemodynamic assessment of untreated STZ-diabetic rats in the present study demonstrates that the cardiomyopathy of this pathophysiological condition is characterized by myocardial contractile dysfunction.

Apart from being a sensitive index of oxidative stress, 8-*iso* PGF_{2α} generated during this process may wield potent vasoconstrictor effects on coronary arteries (43). Therefore, in the present study, we cannot exclude the possibility that this mechanism may be responsible, at least in part, for the decreased cardiac performance observed among the STZ-diabetic animals. We postulate that this may occur through a decrease in subendocardial blood flow. Although the biological effects of 8-*iso* PGF_{2α} suggest that it may act as a mediator of the cellular effects of oxidative stress, a functional role for isoprostanes in cardiac complications of diabetes remains to be established.

Clinical trials examining the use of antioxidants specifically in patients with diabetes is limited. The HOPE (Heart Outcomes Prevention Evaluation) trial (26) and the MICRO-HOPE (44), a sub-study of the HOPE trial, were the two largest trials conducted thus far to investigate the effects of antioxidants on cardiovascular events in diabetic patients. No cardiovascular benefits

for vitamin E in patients with diabetes was found in either trial. The failure of these clinical studies to demonstrate any benefit of vitamin E in preventing cardiovascular disease in diabetic patients is multifactorial. First, there were no measurements using any of the commonly accepted biomarkers to substantiate that oxidative stress was decreased in these studies to an appropriate degree. Second, the human trials to date used endpoints that were not directly related to oxidative stress, but rather gross markers of overall cardiovascular health, such as effect on mortality (45). Finally, these clinical studies included older patients (average age 65.4 years) with established coronary artery disease. It has been suggested that the study populations represented patients in whom the disease states had progressed too far to be amenable to antioxidant intervention (45). A positive effect in an experimental study, such as ours, is most likely due to vitamin E supplementation as a prophylactic treatment (loading the animals with vitamin E before the onset of diabetic cardiovascular disease). In the HOPE (26) and MICRO-HOPE substudy (44), the supplementation was initiated after the clinical diagnosis.

In an effort to determine whether longer duration of vitamin E treatment would prevent cardiovascular disease, the HOPE study was extended (HOPE-The Ongoing Outcomes [HOPE-TOO]) roughly 2.5 years beyond its previously reported (26) 4.5-year mean follow-up. The HOPE-TOO trial investigators actually demonstrated an increased incidence of HF in patients assigned to vitamin E (46). This paradox may be explained by considering that HOPE-TOO study patients had preexisting coronary or peripheral artery disease, stroke, or diabetes mellitus plus at least one other cardiovascular risk factor. Since atherosclerotic vascular disease has been associated with an increase in oxidative stress (31), diabetes mellitus and vasculopathy is a potent combined risk factor for synergistic increases in oxidative stress in these individuals. As a result, these patients were at very high risk for extreme elevations in systemic oxidative stress. The recognition of the ability of vitamin E to become a pro-oxidant in a highly oxidative milieu (47) together with its prolonged administration to HOPE-TOO study patients with known vascular disease and diabetes mellitus may collectively have precluded its antioxidant effects and instead promoted extensive oxidative modification, thereby depressing myocardial function with resultant excess HF. The findings from the HOPE-TOO trial coupled with the beneficial effects of vitamin E supplementation on diabetic cardiomyopathy observed in the present study suggest that the cardioprotective effects of vitamin E may be specific to diabetes mellitus alone, where milder myocardial oxidative stress conditions prevail (48).

Although the present study does not directly address the mechanism by which vitamin E modulates the development of diabetic cardiomyopathy, the fact that vitamin E-supplemented diabetic rats had reduced oxidative stress and an enhanced hemodynamic functioning suggests that the protection afforded by vitamin E may be attributable, in part, to its antioxidant properties. Vitamin E is a lipid-soluble, naturally occurring antioxidant and its presence in biological membranes is thought to represent the major defense system against peroxidation of lipid components. Because vitamin E supplementation produced a reduction in myocardial oxidative stress, its beneficial effect may be due to a combination of its direct antioxidant properties as well as exerting a sparing effect on the remaining myocardial enzymatic antioxidants.

A protective mechanism of vitamin E independent of its direct free radical scavenging effect may also exist. Vitamin E has been shown to decrease platelet adhesion and aggregation (49), promote the inhibition of vitamin K-dependent clotting factors (50), and inhibit the production of nitric oxide (51). These operative mechanisms of action may explain why the use of vitamin E in primary prevention of coronary artery disease has been successful (20,21).

Much of the diastolic dysfunction in diabetes has been attributed to the formation of advanced glycation end-products (AGE's). A common consequence of their formation is covalent cross-

link formation with proteins such as collagen which decrease the compliance of the extracellular matrix (ECM) (52,53). In the myocardium, this may lead to ventricular stiffness (54,55) with resultant impaired diastolic function. Increased activation of the diacylglycerol (DAG)-protein kinase C (PKC) signal transduction pathway has been shown in hearts of STZ-diabetic animals (56) and activation of this pathway has been documented as a mechanism linking AGE's to diabetic complications (57,58). Inhibition of hyperglycemia-induced PKC activation has been reported to produce significant improvements in cardiac dysfunction (59, 60). In that regard, vitamin E has been identified to normalize DAG-PKC activation induced by hyperglycemia (61,62). It is possible that this mechanism may be responsible, at least in part, for the prevention of diastolic dysfunction seen among the STZ-diabetic rats treated with vitamin E in the present study. However, further investigations are needed to verify this hypothesis.

It is important to note that although vitamin E has not been shown in multiple clinical studies to retard the progression of HF (26,44,46,63), all of these studies were performed in patients with established atherosclerotic heart disease and thus it is likely that these individuals HF was of ischemic origin. In contrast, the HF observed in the STZ-diabetic rats in the present study was a result of non-atherogenic cardiomyopathy. These differences in HF etiology are extremely important as vitamin E has been reported to be less effective in mitigating pathophysiological processes related to atherosclerosis as compared to other antioxidants, such as ascorbate (vitamin C) (64). These aspects may partly substantiate the failure of interventional studies using vitamin E supplementation to demonstrate any benefit in preventing HF in patients with advanced coronary artery disease.

Although possible confounding effects of STZ cannot be excluded, the half-life of the drug is ~15 minutes, and it is cleared from the organs within 4 hours (65). Since the present study was conducted at such a late period after STZ treatment (8 weeks post-STZ injection), the results reported herein are likely related to the development of diabetes.

Conclusions

The results of this study demonstrate increased myocardial formation of 8-*iso* PGF_{2α} and GSSG in association with type I diabetic cardiomyopathy. Dietary supplementation with vitamin E was sufficient to reduce myocardial 8-*iso* PGF_{2α} and GSSG levels which resulted in significant protection against cardiac dysfunction induced by type I diabetes. These findings in experimental animals provide compelling evidence in favor of a causal role for myocardial oxidative stress in mediating the pathogenesis of type I diabetic cardiomyopathy. Data obtained in experimental animals must obviously be extrapolated to the clinical arena with caution. Nevertheless, the present findings suggest that vitamin E and other antioxidants may confer cardiovascular benefit in select patients who are diabetic and in greater oxidative stress. Indeed, support for this paradigm has recently been demonstrated in a retrospective analysis of the HOPE study where vitamin E administration to diabetic individuals homozygous for the haptoglobin 2 allele, which confers markedly less antioxidant protection against hemoglobin-induced oxidation (66), was shown to result in a 50% reduction in non-fatal MI and cardiovascular death (67). In all the large scale clinical trials that have examined the effect of antioxidants in diabetes, people with long-standing diabetes (>10 years) and at high risk for cardiovascular events were targeted (26,44). We suggest that it might be fruitful to re-explore the role of antioxidant therapy given immediately after the diagnosis of type I diabetes mellitus to reduce the risk of future cardiovascular complications.

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Figure 1.

Food consumption of control and STZ-induced diabetic rats fed a basal, un-supplemented vitamin E (–Vit E) diet or a diet enriched with vitamin E (+Vit E). Values are mean $\pm$ SEM of 6–8 animals. *Significantly different ($P < 0.05$) from control groups with and without vitamin E supplementation.

Figure 2.

Representative left ventricular (LV) pressure recordings and maximal rate of LV pressure rise and fall ($\pm dP/dt$) obtained from control and STZ-induced diabetic rats with and without vitamin E (Vit E) treatment: **(A)** control rat maintained on a basal, un-supplemented vitamin E ($-Vit E$) diet; **(B)** control rat maintained on a vitamin E-supplemented ($+Vit E$) diet; **(C)** STZ-diabetic rat maintained on a basal, un-supplemented vitamin E ($-Vit E$) diet; **(D)** STZ-diabetic rat maintained on a vitamin E-supplemented ($+Vit E$) diet.

Figure 3.

Myocardial 8-iso PGF_{2α} levels in control and STZ-diabetic animals maintained on a basal, un-supplemented vitamin E (–Vit E) diet or a diet enriched with vitamin E (+Vit E). Data are mean ± SEM from 5 rats, with each assay done in duplicate. *Significantly different (P<0.05) from control groups with and without vitamin E supplementation. #Significantly different (P<0.05) from the un-supplemented diabetic group.

Figure 4. Myocardial levels of oxidized (GSSG) glutathione in control and STZ-diabetic animals maintained on a basal, un-supplemented vitamin E (–Vit E) diet or a diet enriched with vitamin E (+Vit E). Data are mean $\pm$ SEM from 5 rats, with each assay done in duplicate. * $P < 0.05$ vs. control groups with and without vitamin E supplementation. # $P < 0.05$ vs. diabetic group without vitamin E supplementation.

Table 1

Body Weight, Heart Weight, and Ratio of Heart/Body Weight at Eight Weeks After Streptozotocin or Vehicle Injection in Rats

Parameter	Control –Vit E	Control + Vit E	Diabetes –Vit E	Diabetes + Vit E
Body weight (g)	391.6 ± 4.4	394.2 ± 10.3	181.0 ± 9.8 [*]	170.8 ± 15.6 [*]
Heart weight (g)	1.01 ± 0.02	1.14 ± 0.04	0.65 ± 0.04 [*]	0.61 ± 0.04 [*]
Heart/body weight (mg/g)	2.58 ± 0.06	2.88 ± 0.07	3.57 ± 0.09 [*]	3.68 ± 0.10 [*]

Heart weight represents the weight of both ventricles. Values are mean ± SEM of 6–8 animals.

^{*} Significantly different (P<0.05) from control groups with vitamin E (+Vit E) and without vitamin E (–Vit E) supplementation.

Table 2

Effect of Vitamin E Supplementation on Left Ventricular Hemodynamic Parameters in Control and Diabetic Groups of Animals

Parameter	Control – Vit E	Control + Vit E	Diabetes – Vit E	Diabetes + Vit E
LVSP (mm Hg)	129.2 ± 0.9	128.2 ± 3.6	99.6 ± 2.3 [*]	111.5 ± 2.0 ^{*φ}
LVEDP (mm Hg)	0.6 ± 0.5	0.8 ± 0.7	5.1 ± 0.7 [*]	2.8 ± 0.6 ^φ
+dP/dt (mm Hg/s)	8379 ± 98	8136 ± 140	5103 ± 241 [*]	5965 ± 254 ^{*φ}
–dP/dt (mm Hg/s)	7552 ± 287	8024 ± 224	4071 ± 134 [*]	4700 ± 265 [*]
Heart Rate (bpm)	308 ± 6.0	321 ± 4.6	208 ± 9.8 [*]	206 ± 4.6 [*]
MAP (mm Hg)	114.7 ± 3.1	115.8 ± 5.1	87.3 ± 2.9 [*]	101.7 ± 3.4 ^{*φ}

Values are mean ± SEM of 6–8 animals.

^{*} Significantly different (P<0.05) from control groups with vitamin E (+Vit E) and without vitamin E (–Vit E) supplementation.

^φ Significantly different (P<0.05) from the un-supplemented diabetic group.