Diabetes, Oxidative Stress, and Antioxidants: A Review

A. C. Maritim,[1] *R. A. Sanders,*[2] *and J. B. Watkins III*[2]

[1]Moi University, College of Health Sciences, Eldoret, Kenya
[2]Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN, USA; E-mail: *watkins@indiana.edu*

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ABSTRACT: Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus. Changes in oxidative stress biomarkers, including superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione levels, vitamins, lipid peroxidation, nitrite concentration, nonenzymatic glycosylated proteins, and hyperglycemia in diabetes, and their consequences, are discussed in this review. In vivo studies of the effects of various conventional and alternative drugs on these biomarkers are surveyed. There is a need to continue to explore the relationship between free radicals, diabetes, and its complications, and to elucidate the mechanisms by which increased oxidative stress accelerates the development of diabetic complications, in an effort to expand treatment options.
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INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin. Although the etiology of this disease is not well defined, viral infection, autoimmune disease, and environmental factors have been implicated [1–5]. While exogenous insulin and other medications can control many aspects of diabetes, numerous complications affecting the vascular system, kidney, retina, lens, peripheral nerves, and skin are common and are extremely costly in terms of longevity and quality of life.

Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications [6–8]. Diabetes is usually accompanied by increased production of free radicals [7–10] or impaired antioxidant defenses [11–13]. Mechanisms by which increased oxidative stress is involved in the diabetic complications are partly known, including activation of transcription factors, advanced glycated end products (AGEs), and protein kinase C. This review focuses on recent experimental studies of diabetes and drug interventions done within the context of in vivo assay systems. There are also myriad in vitro experiments and clinical studies which deserve a review of their own.

OVERVIEW OF FREE RADICALS AND DIABETIC COMPLICATIONS

Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids, and eventually cell death. Various mechanisms have been suggested to contribute to the formation of these reactive oxygen-free radicals. Glucose oxidation is believed to be the main source of free radicals. In its enediol form, glucose is oxidized in a transition-metal-dependent reaction to an enediol radical anion that is converted into reactive ketoaldehydes and to superoxide anion radicals. The superoxide anion radicals undergo dismutation to hydrogen peroxide, which if not degraded by catalase or glutathione peroxidase, and in the presence of transition metals, can lead to production of extremely reactive hydroxyl radicals [14,15]. Superoxide anion radicals can also react with nitric oxide to form reactive peroxynitrite radicals [11,16]. Hyperglycemia is also found to promote lipid peroxidation of low density lipoprotein (LDL) by a superoxide-dependent pathway resulting in the generation of free radicals [17,18]. Another important

Correspondence to: J. B. Watkins III.
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source of free radicals in diabetes is the interaction of glucose with proteins leading to the formation of an Amadori product and then advanced glycation endproducts (AGEs) [19,20]. These AGEs, via their receptors (RAGEs), inactivate enzymes and alter their structures and functions [21], promote free radical formation [7,8], and quench and block antiproliferative effects of nitric oxide [22,23]. By increasing intracellular oxidative stress, AGEs activate the transcription factor NF-κB, thus promoting up-regulation of various NF-κB controlled target genes [24]. NF-κB enhances production of nitric oxide, which is believed to be a mediator of islet beta cell damage.

Considerable evidence also implicates activation of the sorbitol pathway by glucose as a component in the pathogenesis of diabetic complications, for example, in lens cataract formation or peripheral neuropathy [25–27]. Efforts to understand cataract formation have provoked various hypotheses. In the aldose reductase osmotic hypothesis, accumulation of polyols initiates lenticular osmotic changes. In addition, oxidative stress is linked to decreased glutathione levels and depletion of NADPH levels [28,29]. Alternatively, increased sorbitol dehydrogenase activity is associated with altered NAD^+ levels [30], which results in protein modification by nonenzymatic glycosylation of lens proteins [31,32].

Mechanisms linking the changes in diabetic neuropathy and induced sorbitol pathway are not well delineated. One possible mechanism, metabolic imbalances in the neural tissues, has been implicated in impaired neurotrophism [33–35], neurotransmission changes [36–38], Schwann cell injury [39,40], and axonopathy [41,42].

OVERVIEW OF ANTIOXIDANTS

While on the one hand hyperglycemia engenders free radicals, on the other hand it also impairs the endogenous antioxidant defense system in many ways during diabetes [12]. Antioxidant defense mechanisms involve both enzymatic and nonenzymatic strategies. Common antioxidants include the vitamins A, C, and E, glutathione, and the enzymes superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase. Other antioxidants include α-lipoic acid, mixed carotenoids, coenzyme Q_{10}, several bioflavonoids, antioxidant minerals (copper, zinc, manganese, and selenium), and the cofactors (folic acid, vitamins B_1, B_2, B_6, B_{12}). They work in synergy with each other and against different types of free radicals. Vitamin E suppresses the propagation of lipid peroxidation; vitamin C, with vitamin E, inhibits hydroperoxide formation; metal complexing agents, such as penicillamine, bind transition metals involved in some reactions in lipid peroxidation [43] and inhibit Fenton- and Haber-Weiss-type reactions; vitamins A and E scavenge free radicals [8,11,44–47].

Extensive studies of pharmacological interventions based on biological antioxidants have been carried out since the last review by Oberley [48]. Discrepancies in observed biomarkers for oxidative stress continue to be seen in the present review, especially in the activities of SOD, catalase, and glutathione peroxidase in experimentally diabetic animals. Decreased levels of glutathione and elevated concentrations of thiobarbituric acid reactants are consistently observed in diabetes. In addition, changes in nitric oxide and glycated proteins are also seen in diabetes. The effects of antioxidants on these biomarkers for oxidative stress are summarized here after.

BIOMARKERS OF OXIDATIVE STRESS: IN VIVO DIABETES STUDIES

Lipid Peroxidation

Hydroperoxides have toxic effects on cells both directly and through degradation to highly toxic hydroxyl radicals. They may also react with transition metals like iron or copper to form stable aldehydes such as malondialdehydes that will damage cell membranes. Peroxyl radicals can remove hydrogen from lipids, producing hydroperoxides that further propagate the free-radical pathway [11].

Induction of diabetes in rats with streptozotocin (STZ) or alloxan uniformly results in an increase in thiobarbituric acid reactive substances (TBARS) (Table 1), an indirect evidence of intensified free-radical production. Preventing the formation of hydroxyl radicals would be an efficient means to reduce hydroxyl-induced damage, and several compounds have been tested as antioxidants in diabetic animals with varying success. For example, the increase in TBARS associated with diabetes is prevented by treatment with nicotinamide [61], boldine [62], melatonin [45,49,63], aspirin [74], L-arginine or sodium nitroprusside [67], probucol [51], α-lipoic acid [71,77], aminoguanidine [69], captopril, enalapril [65], or nitecapone [66], if this treatment is given before or immediately after the diabetogen.

Even after diabetes is established, the buildup of TBARS may be reversed by treatment with combined vitamins C, E, and β-carotene [78], melatonin [58], gemfibrozil [53], probucol [52,80], and vitamin E [80]. Dietary supplementation with α-lipoic acid, evening primrose oil or sunflower oil lowers plasma lipids and hemostatic risk factors [81].

These normalization effects are seen in kidney [58,59,62,65,66,78], liver [58–62,64,74], heart [51–53,77], brain [49], intestine [58], lung [60], pancreas [45,61,62],

TABLE 1. Effect of Diabetogen and Diabetogen Plus Antioxidant on the Concentration of Thiobarbituric Acid Reactive Substances (TBARS)

	Diabetogen	*Animal*	*Kidney*	*Liver*	*Heart*	*Brain*	*Other*
Pierrefiche et al. (1993) [49]	ALX	♂ Mice				⇑	
Melatonin, 100–450 mg/kg, i.p.	40 mg/kg, i.v.					N	
Thompson and McNeill (1993) [50]	STZ	♂ Wistar rats		⇑			
Vanadyl SO_4, 1–1.25 mg/mL in water				⇑⇑			
Kaul et al. (1995) [51]	STZ	♂ SD rats			⇑		
Probucol, 10 mg/kg, i.p. on day 1 after STZ for 4 weeks	65 mg/kg, i.v.				⇓ But not N		
Kaul et al. (1996) [52]	STZ	♂ SD rats			⇑		
Probucol 10 mg/kg, i.p. weeks 5–8 after STZ	65 mg/kg, i.v.				⇓ But not N		
Ozansoy et al. (2000) [53]	STZ	♂ Wistar rats			⇑ (Aorta)		⇑ (Plasma)
Gemfibrozil 100 mg/kg, p.o. weeks12–14 of induced diabetes	45 mg/kg, i.p.				N		N
Rauscher et al. (2001) [54]	STZ	♂ SD rats		⇑			
Coenzyme Q_{10} 10 mg/kg, i.p. weeks 5–6 post STZ	100 mg/kg, i.p.			O			
Rauscher et al. (2001) [55]	STZ	♂ SD rats		⇑			
Isoeugenol 10 mg/kg, i.p. weeks 5–6 post STZ	100 mg/kg, i.p.			O			
Rauscher et al. (2000) [56]	STZ	♂ SD rats		⇑			
PNU-104067F or PNU-74389G 10 mg/kg, i.p. weeks 5–6 post STZ	100 mg/kg, i.p.			O			
Rauscher et al. (2000) [57]	STZ	♂ SD rats		⇑			
Piperine 10 mg/kg, i.p. weeks 5–6 post STZ	100 mg/kg, i.p.			O			
Maritim et al. (1999) [58]	STZ	♀SD rats	⇑	⇑			⇑ (Intestine)
Melatonin 10 mg/kg, i.p. on days 30–34 post STZ	50 mg/kg, i.v.		N	N			N
Aragno et al. (1999) [59]	STZ	♂ Wistar rats	⇑	⇑		⇑	
DHEA 4 mg/kg orally for 3 weeks	50 mg/kg		N	N		N	
Cinar et al. (1999) [60]	STZ	♂ Wistar rats		⇑			⇑ (Lung)
Vitamin E supplement 1000 mg/kg chow for 12 weeks	50 mg/kg			N			N
Melo et al. (2000) [61]	STZ	♂ Wistar rats		⇑			⇑ (Pancreas)
Nicotinamide 500 mg/kg diet for 1–4 weeks prior to STZ	40 mg/kg, i.v.			N			N
Jang et al. (2000) [62]	STZ	♂ SD rats	⇑	⇑			⇑ (Pancreas)
Boldine 100 mg/kg/day in water for 8 weeks immediately after STZ	80 mg/kg, i.p.		O	N			N
Montilla et al. (1998) [63]	STZ	♀ Wistar rats					⇑ (Plasma, RBCs)
Melatonin 100 and 200 μg/kg, i.p. -3 days to 8 weeks of STZ	60 mg/kg, i.p.						N
El-Missiry and El-Gindy (2000) [64]	ALX	♂ Wistar rats		⇑			
Oil of *Eruca sativa* seeds 0.06 mL/kg orally	100 mg/kg			N			
Kedziora-Kornatowski et al. (2000) [65]	STZ	♂ Wistar rats	⇑				
Captopril 2 mg/kg or enalapril 1 mg/kg in water for 6 and 12 weeks	65 mg/kg, i.p.		N				
Lal et al. (2000) [66]	STZ	♂ SD rats	⇑				
Nitecapone 30 mg/kg aq. soln. 2× day or via gavage 25 μg/mL	70 mg/kg, i.p.		N				
Mohan and Das (1998) [67]	ALX	♂ Wistar rats					⇑ (Plasma)
L-arginine 50 mg in 0.5 mL NaCl pre- and simultaneous with ALX	75 mg/kg/day * 5 days						N
Sodium nitroprusside 2–10 μg pre- and simultaneous with ALX							N
El-Khatib et al. (2001) [68]	STZ	♂ Wistar rats					⇑ (Plasma)
Aminoguanidine 100 mg/kg, i.p. for 14 days	65 mg/kg, i.p.						N

Continued

TABLE 1. Continued

	Diabetogen	*Animal*	*Kidney*	*Liver*	*Heart*	*Brain*	*Other*
Desferrioxamine 50 mg/kg, i.p. for 14 days							N
Abdel-Wahab and Abd-Allah (2000) [45]	STZ	♂ mice					⇑ (Pancreas)
Melatonin 5 mg/kg orally, -3 and 15 days after STZ injection	60 mg/kg/day, i.p. * 3 days						N
Melatonin + desferrioxamine 250 mg/kg, p.o., -3 and 15 days after STZ							N
Kedsiora-Kornatowski et al. (1998) [69]	STZ	♂ Wistar rats					⇑ (RBC)
Aminoguanidine 1 g/L in water for 6 and 12 weeks	65 mg/kg, i.p.						N
Obrosova et al. (1999) [70]	STZ 55 mg/kg, i.p.	♂ SD rats					⇑ (Precataract lens)
Taurine 5% in feed for 3 weeks							N
Obrosova et al. (2000) [71]	STZ	♂ Wistar rats					⇑ (Retina)
α-Lipoic acid 100 mg/kg, i.p. for 6 weeks starting 48 h after STZ	55 mg/kg, i.p.						N
van Dam et al. (2001) [72]	STZ	♂ Wistar rats					⇑ (Plasma)
α-Lipoic acid (different doses)	i.p.						N
Sailaja Devi and Das (2000) [73]	ALX	♂ Wistar rats		⇑			⇑ (Plasma)
Melatonin 200 μg/rat, p.o. with ALX + 7 weeks	75 mg/kg, i.p.			⇓			N
Caballero et al. (2000) [74]	STZ	♂ CF1 mice		⇑			
Aspirin 0.16% w/w in diet, 30 min after STZ injection	200 mg/kg, i.p.			N			
Sanders et al. (2001) [75]	STZ	♂ SD rats		⇑			
Quercetin 10 mg/kg/day, i.p. weeks 5–6 post STZ	100 mg/kg, i.p.			⇑⇑			
Obrosova et al. (1999) [76]	STZ	♂ Wistar rats					⇑ (Nerve)
SDI-157 100 mg/kg in water 48 h to 3 weeks after STZ	55 mg/kg, i.p.						⇑⇑
Kocak et al. (2000) [77]	STZ	♂ Wistar rats			⇑		
α-Lipoic acid 50 mg/kg/day, i.p. for 6 weeks	55 mg/kg, i.p.				N		
Mekinova et al. (1995) [78]	STZ	♂ Wistar rats	⇑				
Vitamins C, E, and β-carotene p.o. 8 days after STZ + 8 weeks	45 mg/kg, i.v.		N				
Altavilla et al. (2001) [79]		Diabetic mice C57BL/ Ksdb +/db +					⇑ (Wound dienes)
Raxofelast 15 mg/kg/day, i.p. for 3, 6, 12 days							N

Diabetogens alloxan (ALX) or streptozotocin (STZ), administered to mice or Wistar or Sprague-Dawley (SD) rats, produced increases (⇑) or decreases (⇓) from normal levels of TBARS as indicated in the top line for each study. Dose and route of diabetogen administration is indicated in the second line of column 2, and the asterisk in line 3 indicates diabetogen treatment continued for the specified number of days. Treatment with antioxidant chemicals produced no effect (O), further increase (⇑⇑), or normal levels (N) of TBARS at the specified time, as indicated in lower line(s) of each study.

plasma [53,63,67,68,72], red blood cells [63,69], lens [70], and retina [71]. In addition, increased lipid peroxidation in genetically diabetic C57BL/Ksdb+/db+ mice, as measured by conjugated dienes at wound sites, returns to normal levels after raxofelast treatment [79].

In contrast, both basal and iron-stimulated TBARS levels are significantly elevated in livers of rats treated with vanadyl sulfate compared to untreated STZ-induced diabetic rats, highlighting the importance of using multiple indicators of peroxidative change [50]. Similarly, quercetin [75] and the sorbitol dehydrogenase inhibitor SDI-157 [76] exacerbate the increased TBARS concentrations in livers [75] and nerves [76] of untreated diabetic rats. On the other hand treatment with coenzyme Q_{10} [54], piperine [57], isoeugenol [55], or experimental antioxidants PNU-104067F or PNU-74389G [56], results in no change in lipid peroxidation in liver, kidney, heart, and brain of diabetic rats.

Glutathione Levels

Reduced glutathione is a major intracellular redox buffer that may approach concentrations up to 10 mM [82]. Glutathione functions as a direct free-radical scavenger, as a cosubstrate for glutathione peroxidase

activity, and as a cofactor for many enzymes, and forms conjugates in endo- and xenobiotic reactions [83,84].

Table 2 summarizes recent studies of the effects of various antioxidants on glutathione concentrations. Glutathione concentration is found to be decreased in the liver [50,54–59,61,64,75], kidney [59], pancreas [45], plasma [63,67], red blood cells [63], nerve [76], and precataractous lens [70] of chemically induced diabetic animals. However, there is also some contradictory evidence of increased glutathione concentration in diabetic rat kidney [78] and lens [85].

Levels of glutathione are reported to be normalized by vanadyl [50], dehydroepiandrosterone (DHEA) [59], oil of *Eruca sativa* seeds [64], nicotinamide [61], L-arginine or nitroprusside [67], melatonin [63], and melatonin plus desferrioxamine [45] when these antioxidants are given prior to or at the same time as the diabetogen. However, antioxidants that fail to reverse the effects of established diabetes on glutathione levels include coenzyme Q_{10} [54], quercetin [75], piperine [57], isoeugenol [55], PNU-104067F or PNU-74389G [56], DHEA [59], melatonin [58], and taurine [70].

The increase in renal glutathione levels in diabetic Wistar rats is normalized by simultaneous treatment with vitamin C, vitamin E, and β-carotene [78]. Sand rats modeling both type I and type II diabetes had increased levels of glutathione in lens, which were normalized by treatment with α-lipoic acid [85].

Glutathione Peroxidase and Glutathione Reductase

Glutathione peroxidase and reductase are two enzymes that are found in the cytoplasm, mitochondria, and nucleus. Glutathione peroxidase metabolizes hydrogen peroxide to water by using reduced glutathione as a hydrogen donor [86,87]. Glutathione disulfide is recycled back to glutathione by glutathione reductase, using the cofactor NADPH generated by glucose 6-phosphate dehydrogenase. Investigations into the effects of various drugs on these two enzymes in the tissues of diabetic animals are summarized in Table 3.

There is not total agreement about the effects of diabetes on the activities of these enzymes. However, glutathione peroxidase activity is seen to be elevated in liver [54–57,59,75], kidney [54,55,57,59,65,75,78], aorta [77], pancreas [62], blood [67–69], and red blood cells [73], whereas decreased activity was seen in heart [51,52] and retina [71].

Diabetes-induced alterations in glutathione peroxidase activity are reversed by treatment with probucol [51,52], DHEA [59], combined vitamins C, E, and β-carotene [78], quercetin (in liver and brain, though not in kidney or heart) [75], coenzyme Q_{10} and isoeugenol (only in liver) [54,55], piperine (in kidney) [57], boldine [62], aminoguanidine [68], desferioxamine [68], L-arginine and nitrooprusside [67], captopril and enalapril [69], melatonin [73], and α-lipoic acid [77]. Altered enzyme activity in diabetic animals is not restored to normal levels by α-lipoic acid in retina [71], boldine in kidney [62], quercetin [75] or coenzyme Q_{10} [54] in heart and kidney, or piperine in heart and liver [57]. It is interesting to note that all these studies instituted antioxidant treatment after diabetes was well established, as opposed to prior to or simultaneously with the diabetogen. Aminoguanidine treatment attenuates erythrocyte glutathione peroxidase activity, exceeding control values after both 6 and 12 weeks of induced diabetes [69].

Activity of glutathione reductase, which regenerates cellular glutathione, is reduced in retina [71] and plasma [67] but increased in heart [54,55,57,75] of diabetic animals. None of these effects is reversed by treatment with antioxidants, including α-lipoic acid, quercetin, piperine, isoeugenol, coenzyme Q_{10}, L-arginine, or nitroprusside.

Catalase

Catalase, located in peroxisomes, decomposes hydrogen peroxide to water and oxygen [88]. Documented changes in catalase activity in chemically induced diabetic animals are given in Table 4. For example, catalase activity is consistently found to be elevated in heart [51,52,54,55,57,75,89] and aorta [53,77], as well as brain [59] of diabetic rats. In contrast to decreased renal [58,65,78], hepatic [54,58,75] and red blood cell [69] catalase activity, catalase activity in liver [59,74] and kidney [59] of diabetic animals is increased.

These alterations of catalase activity due to diabetes are normalized by treatment with captopril [65], aminoguanidine [69], melatonin (in liver) [58], acetylsalicylic acid [74], DHEA [59], probucol [51,52], α-lipoic acid [77], and stobadine [89], all of which were administered before or at the same time as the diabetogen. By contrast, treatment of established diabetes of 4 weeks or more does not reverse or normalize diabetic effects. For example, no reversals are seen after treatment with melatonin [58], quercetin [75], coenzyme Q_{10} [54], piperine [57], isoeugenol [55], gemfibrozil [53], or combined vitamin C, vitamin E, and β-carotene [78]. Finally, effects of diabetes on cardiac catalase activity are exacerbated by treatment with quercetin [75] or coenzyme Q_{10} [54].

Superoxide Dismutase (SOD)

Isoforms of SOD are variously located within the cell. CuZn-SOD is found in both the cytoplasm and the

TABLE 2. Effect of Diabetogen and Diabetogen Plus Antioxidant on the Concentration of Reduced Glutathione (GSH)

	Diabetogen	*Animal*	*Kidney*	*Liver*	*Heart*	*Brain*	*Other*
Thompson and McNeill (1993) [50]	STZ	♂ Wistar rats		⇓			
Vanadyl SO_4 1–1.25 mg/mL in water				N			
Rauscher et al. (2001) [54]	STZ	♂ SD rats		⇓			
Coenzyme Q_{10} 10 mg/kg, i.p. weeks 5–6 post STZ	100 mg/kg, i.p.			O			
Rauscher et al. (2001) [55]	STZ	♂ SD rats		⇓			
Isoeugenol 10 mg/kg, i.p. weeks 5–6 post STZ	100 mg/kg, i.p.			O			
Rauscher et al. (2000) [57]	STZ	♂ SD rats		⇓			
Piperine 10 mg/kg, i.p. weeks 5–6 post STZ	100 mg/kg, i.p.			O			
Rauscher et al. (2000) [56]	STZ	♂ SD rats		⇓			
PNU-104067F or PNU-74389G 10 mg/kg, i.p. weeks 5–6 post STZ	100 mg/kg, i.p.			O			
Sanders et al. (2001) [75]	STZ	♂ SD rats		⇓			
Quercetin 10 mg/kg/day, i.p. weeks 5–6 post STZ	100 mg/kg, i.p.			O			
Aragno et al. (1999) [59]	STZ	♂ Wistar rats	⇓	⇓			
DHEA 4 mg/kg orally for 3 weeks	50 mg/kg		N	N			
El-Missiry and El-Gindy (2000) [64]	ALX	♂ Wistar rats		⇓			
Oil of *Eruca sativa* seeds 0.06 mL/kg orally	100 mg/kg			N			
Maritim et al. (1999) [58]	STZ	♀ SD rats	O	⇓			O
Melatonin 10 mg/kg, i.p. on days 30–34 post STZ	50 mg/kg, i.v.		O	O			O (Intestines)
Mohan and Das (1998) [67]	ALX	♂ Wistar rats					⇓ (Plasma)
L-arginine 50 mg in 0.5 mL NaCl pre- and simultaneously with ALX	75 mg/kg/day * 5 days						N
Na nitroprusside 2–10 μg pre- and simultaneously with ALX							N
Montilla et al. (1998) [63]	STZ	♀ Wistar rats					⇓ (RBC, Plasma)
Melatonin 100 and 200 μg/kg, i.p. -3 days to 8 weeks of STZ	60 mg/kg, i.p.						N
Abdel-Wahab and Abd-Allah (2000) [45]	STZ	♂ mice					⇓ (Pancreas)
Melatonin 5 mg/kg orally, -3 and 15 days after STZ injection	60 mg/kg/day i.p. * 3 days						N
Melatonin + desferrioxamine 250 mg/kg, p.o., -3 and 15 days after STZ							N
Melo et al. (2000) [61]	STZ	♂ Wistar rats		⇓			
Nicotinamide 500 mg/kg diet for 4 weeks prior STZ injection	40 mg/kg, i.v.			N			
Obrosova et al. (1999) [76]	STZ	♂ Wistar rats					⇓ (Nerve)
SDI-157 100 mg/kg in water 48 h to 3 weeks after STZ	55 mg/kg, i.p.						⇓
Obrosova et al. (1999) [70]	STZ 55 mg/kg, i.p.	♂ SD rats					⇓ (Precataract lens)
Taurine 1% in diet for 3 weeks							O
Taurine 5% in diet for 3 weeks							O
Mekinova et al. (1995) [78]	STZ	♂ Wistar rats	⇑				
Vitamins C, E, and β-carotene p.o. in weeks 2–8 after STZ	45 mg/kg, i.v.		N				
Borenshtein et al. (2001) [85]		Sand rats (Type I and ll DM)					⇓ Lens
α-Lipoic acid, γ-linolenic acid i.p.							N

Diabetogens alloxan (ALX) or streptozotocin (STZ), administered to mice or Wistar and/or Sprague-Dawley (SD) rats, produced increases (⇑) or decreases (⇓) from normal concentrations of GSH as indicated in top line for each study. Dose and route of diabetogen administration is indicated in the second line of column 2, and the asterisk in line 3 indicates diabetogen treatment continued for the specified number of days. Treatment with antioxidant chemicals produced no effect (O) or normal levels (N) of GSH at the specified time, as indicated in lower line(s) of each study.

TABLE 3. Effect of Diabetogen and Diabetogen Plus Antioxidant on the Activity of Glutathione Peroxidase

	Diabetogen	*Animal*	*Kidney*	*Liver*	*Heart*	*Brain*	*Other*
Kaul et al. (1995, 1996) [51,52]	STZ	♂ SD rats			⇓		
Probucol 10 mg/kg, i.p., on day 1 after STZ + 4 weeks	65 mg/kg, i.v.				⇑ But not N		
Probucol 10 mg/kg, i.p., weeks 5–8 post STZ					N		
Aragno et al. (1999) [59]	STZ	♂ Wistar rats	⇓	⇓			
DHEA 4 mg/kg orally for 3 weeks	50 mg/kg		N	N			
Obrosova et al. (2000) [71]	STZ	♂ Wistar rats					⇓ (Retina)
α-Lipoic acid 100 mg/kg, i.p., starting 48 h after STZ injection	55 mg/kg, i.p.						O
Mekinova et al. (1995) [78]	STZ	♂ Wistar rats	⇑				
Vitamins C, E, and β-carotene p.o. weeks 2–8 after STZ	45 mg/kg, i.v.		N				
Sanders et al. (2001) [75]	STZ	♂ SD rats	⇑	⇑	⇑	⇑	
Quercetin 10 mg/kg/day, i.p. weeks 5–6 post STZ	100 mg/kg, i.p.		O	N	O	O	
Rauscher et al. (2001) [54]	STZ	♂ SD rats	⇑	⇑	⇑	⇑	
Coenzyme Q_{10} 10 mg/kg, i.p. weeks 5–6 post STZ	100 mg/kg, i.p.		O	N	O	O	
Rauscher et al. (2000) [57]	STZ	♂ SD rats	⇑	⇑	⇑	⇑	
Piperine 10 mg/kg, i.p. weeks 5–6 post STZ	100 mg/kg, i.p.		N	O	O		
Rauscher et al. (2001) [55]	STZ	♂ SD rats	⇑	⇑	⇑	⇑	
Isoeugenol 10 mg/kg, i.p. weeks 5–6 post STZ	100 mg/kg, i.p.		O	N	O	O	
Rauscher et al. (2000) [56]	STZ	♂ SD rats	O	O	O	O	
PNU-104067F or PNU-74389G 10 mg/kg, i.p. weeks 5–6 post STZ	100 mg/kg, i.p.		O	⇓	⇑	⇑	
Jang et al. (2000) [62]	STZ	♂ SD rats	⇑	⇓			⇑ (Pancreas)
Boldine 100 mg/kg/day in water for 8 weeks immediately after STZ	80 mg/kg, i.p.		O	N			N
El-Khatib et al. (2001) [68]	STZ	♂ Wistar rats					⇑ (Blood)
Aminoguanidine 100 mg/kg, i.p. for 14 days	65 mg/kg, i.p.						N
Desferrioxamine 50 mg/kg, i.p. for 14 days							N
Mohan and Das (1998) [67]	ALX	♂ Wistar rats					⇑ (Plasma)
L-arginine 50 mg in 0.5 mL NaCl pre- and simultaneous with ALX	75 mg/kg/day *5 days						N
Sodium nitroprusside 2–10 μg pre- and simultaneous with ALX							N
Kedziova-Kornatowska et al. (1998) [69]	STZ	♂ Wistar rats					⇑ (RBCs)
Aminoguanidine 1 g/L in water for 6 and 12 weeks	65 mg/kg , i.p.						N (12 weeks)
Kedziova-Kornatowska et al. (2000) [65]	STZ	♂ Wistar rats	⇑				
Captopril 2 mg/kg or enalapril 1 mg/kg in water for 6 and 12 weeks	65 mg/kg, i.p.		N				
Maritim et al. (1999) [58]	STZ	♀ SD rats	O	O			O (Spleen)
Melatonin 10 mg/kg, i.p. on days 30–34 post STZ	50 mg/kg, i.v.			⇓			
Sailaja Devi and Das (2000) [73]	ALX	♂ Wistar rats					⇑ (Plasma)
Melatonin 200 μg/rat p.o. with ALX + 7 weeks	75 mg/kg, i.p.						N
Kocak et al. (2000) [77]	STZ	♂ Wistar rats			⇑ (Aorta)		
α-Lipoic acid 50 mg/kg/day i.p. for 6 weeks	55 mg/kg, i.p.				N		
Ozansoy et al. (2000) [53]	STZ	♂ Wistar rats			O (Aorta)		
Gemfibrozil 100 mg/kg p.o. weeks 12–14 of induced diabetes	45 mg/kg, i.p.				O		

Diabetogens alloxan (ALX) or streptozotocin (STZ), administered to Wistar or Sprague-Dawley (SD) rats, produced increases (⇑) or decreases (⇓) from normal activities of glutathione peroxidase as indicated in the top line for each study. Dose and route of diabetogen administration is indicated in the second line of column 2, and the asterisk in line 3 indicates diabetogen treatment continued for the specified number of days. Treatment with antioxidant chemicals produced no effect (O), increase (⇑), decrease (⇓), or normal activities (N) of glutathione peroxidase at the specified time, as indicated on the lower line(s) for each study.

TABLE 4. Effect of Diabetogen and Diabetogen Plus Antioxidant on the Activity of Superoxide Dismutase

	Diabetogen	*Animal*	*Kidney*	*Liver*	*Heart*	*Brain*	*Other*
Kaul et al. (1995) [51]	STZ	♂ SD rats			⇓		
Probucol 10 mg/kg, i.p. for 4 weeks	65 mg/kg, i.v.				N		
Kaul et al. (1996) [52]	STZ	♂ SD rats			⇓		
Probucol 10 mg/kg, i.p. weeks 5–8 after STZ	65 mg/kg, i.v.				N		
Mohan and Das (1998) [67]	ALX	♂ Wistar rats					⇓ (Plasma)
L-arginine 50 mg in 0.5 mL NaCl pre- and simultaneous with ALX	75 mg/kg/day * 5 days						○
Sodium nitroprusside 2–10 μg pre- and simultaneous with ALX							N
Kedziora-Kornatowski et al. (1998) [69]	STZ	♂ Wistar rats					⇓ (RBC)
Aminoguanidine (1 g/L in water, 6 and 12 weeks)	65 mg/kg, i.p.						○
Aminoguanidine (1 g/L in water, 6 and 12 weeks)							N
Kedziora-Kornatowski et al. (2000) [65]	STZ	i.p. ♂ Wistar rats	⇓				
Captopril (2 mg/kg in water, 6 and 12 weeks)	65 mg/kg		N				
Enalapril (1 mg/kg in water, 6 and 12 weeks)			○				
Maritim et al. (1999) [58]	STZ	♀ SD rats	○	⇓			
Melatonin 10 mg/kg, i.p. days 30–34 post STZ	50 mg/kg, i.v.		○	○			
Obrosova et al. (2000) [71]	STZ	♂ Wistar rats					⇓ (Retina)
α-Lipoic acid (100 mg/kg/day) 6 weeks starting 48 h post STZ	55 mg/kg, i.p.						N
Aragno et al. (1999) [59]	STZ	♂ Wistar rats	⇓	⇓			
DHEA 4 mg/kg orally for 3 weeks	50 mg/kg		N	N			
Sailaja Devi and Das (2000) [73]	ALX	♂ Wistar rats					⇑ (Plasma)
Melatonin 200 μg/rat p.o. with ALX + 7 weeks	75 mg/kg, i.p.						N
Rauscher et al. (2001) [54]	STZ	♂ SD rats	⇑				
Coenzyme Q_{10} 10 mg/kg, i.p. in weeks 5 and 6 post STZ	100 mg/kg, i.p.		N				
Rauscher et al. (2000) [57]	STZ	♂ SD rats	⇑				
Piperine 10 mg/kg, i.p. in weeks 5 and 6 post STZ	100 mg/kg, i.p.		N				
Jang et al. (2000) [62]	STZ	♂ SD rats	⇑	⇑			⇑ (Pancreas)
Boldine 100 mg/kg/day in water for 8 weeks immediately after STZ	80 mg/kg, i.p.		N	○			N
Lal et al. (2000) [66]	STZ	♂ SD rats	⇑				
Nitecapone 30 mg/kg aq. soln. 2× day or via gavage 25 μg/mL	70 mg/kg, i.p.		N				
El-Khatib et al. (2001) [68]	STZ	♂ Wistar rats					⇑ (RBC)
Aminoguanidine 100 mg/kg, i.p. for 14 days	65 mg/kg, i.p.						○
Desferrioxamine 50 mg/kg, i.p. for 14 days							○
Ozansoy et al. (2000) [53]	STZ	♂ Wistar rats			○ (Aorta)		
Gemfibrozil 100 mg/kg p.o. weeks 12–14 of induced diabetes	45 mg/kg, i.p.				○		
Mekinova et al. (1995) [78]	STZ	♂ Wistar rats		○			
Vitamin C, E, and β-carotene p.o. weeks 2–8 after STZ	45 mg/kg, i.v.			⇑			
Kocak et al. (2000) [77]	STZ	♂ Wistar rats			○ (Aorta)		
α-Lipoic acid 50 mg/kg/day i.p. for 6 weeks	55 mg/kg, i.p.				○		
Stefek et al. (2000) [89]	STZ	♂ Wistar rats			⇑		
Stobadine 0.05% w/w for 32 days					N		

Diabetogens alloxan (ALX) or streptozotocin (STZ), administered to Wistar or Sprague-Dawley (SD) rats, produced increases (⇑) or decreases (⇓) from normal activity of superoxide dismutase as indicated in the top line for each study. Dose and route of diabetogen administration is indicated in the second line of column 2, and the asterisk in line 3 indicates diabetogen treatment continued for the specified number of days. Treatment with antioxidant chemicals produced no effect (○), increased (⇑), or normal activities (N) of superoxide dismutase at the specified time, as indicated in the lower line(s) of each study.

nucleus. Mn-SOD is confined to the mitochondria, but can be released into extracellular space [90]. SOD converts superoxide anion radicals produced in the body to hydrogen peroxide, thereby reducing the likelihood of superoxide anion interacting with nitric oxide to form reactive peroxynitrite. Changes in SOD activity in the tissues of chemically induced diabetic animals are surveyed and summarized in Table 5.

The effect of diabetes on the activity of SOD is erratic, with no discernable pattern based on gender or species of animal, or duration of diabetes, or tissue studied. Renal activity, for example, is within normal levels at 3 [59] and 6 weeks [58] after STZ, lower than normal at 6 weeks [54,75] post-STZ, but also elevated after 6 or 12 weeks of diabetes [65]. In liver, SOD activity is depressed by the third [59] or fourth week [58] of diabetes, but is either normal [78] or elevated [62] 8 weeks after STZ. Kaul et al. [51,52] found cardiac SOD activity decreased after 4 or 8 weeks of diabetes, but Stefek et al. [89] reported elevated cardiac activity at 32 weeks, and activity in aorta seems to be unaffected by diabetes [53,77]. Likewise, activity may be elevated [68,73] or decreased [69] in red blood cells, decreased in retina [71] and plasma [67], and increased in pancreas [62].

Alterations of SOD activity in diabetic animals are normalized by probucol [51,52], captopril [69], DHEA [59], α-lipoic acid [71], melatonin [73], boldine [62], nitecapone [66], and stobadine [89], all of which were administered prior to or concomitant with the diabetogen. When treatment is initiated in animals with well-established diabetes, coenzyme Q_{10} [54] and piperine

TABLE 5. Effect of Diabetogen and Diabetogen Plus Antioxidant on the Activity of Catalase

	Diabetogen	*Animal*	*Kidney*	*Liver*	*Heart*	*Brain*	*Other*
Kedziova-Kornatowska et al. (1998) [69]	STZ	♂ Wistar rats					⇓
Aminoguanidine (1 g/L in water) for 6 and 12 weeks post STZ	65 mg, i.p.						N-(RBC)
Kedziova-Kornatowska et al. (2000) [65]	STZ	♂ Wistar rats	⇓				
Captopril 2 mg/kg in water 6 and 12 weeks post STZ	65 mg, i.p.		N				
Enalapril 1 mg/kg in water 6 and 12 weeks post STZ			N				
Maritim et al. (1999) [58]	STZ	♀ SD rats	⇓	⇓			
Melatonin 10 mg/kg, i.p. days 30–34 post STZ	50 mg/kg, i.v.		○	N			
Sanders et al. (2001) [75]	STZ	♂ SD rats		⇓	⇑		
Quercetin 10 mg/kg/day, i.p. weeks 5 and 6 post STZ	100 mg/kg, i.p.			○	⇑⇑		
Rauscher et al. (2001) [54]	STZ	♂ SD rats		⇓	⇑		
Coenzyme Q_{10} 10 mg/kg, i.p. weeks 5 and 6 post STZ	100 mg/kg, i.p.			○	⇑⇑		
Mekinova et al. (1995) [78]	STZ	♂ Wistar rats	⇓				
Vitamin C, E, and β-carotene p.o. weeks 2–8 after STZ	45 mg/kg, i.v.		○				
Caballero et al. (2000) [74]	STZ	♂ CF1 mice		⇑			
Acetylsalicylic acid 0.16% w/w in diet for 7 and 45 days post STZ	200 mg, i.p.			N (15d)			
Aragno et al. (1999) [59]	STZ	♂ Wistar rats				⇑	
DHEA 4 mg/kg orally for 3 weeks	50 mg/kg					N	
Kaul et al. (1995, 1996) [51,52]	STZ	♂ SD rats			⇑		
Probucol 10 mg/kg, i.p. on day 1 thru 4 weeks after STZ	65 mg, i.v.				N		
Probucol given weeks 5–8 post STZ					N		
Ozansoy et al. (2000) [53]	STZ	♂ Wistar rats			⇑ (Aorta)		
Gemfibrozil 100 mg/kg p.o. weeks 12–14 of induced diabetes	45 mg/kg, i.p.				○		
Kocak et al. (2000) [77]	STZ	♂ Wistar rats			⇑		
α-Lipoic acid 50 mg/kg, i.p. for 6 weeks	55 mg, i.p.				N (Aorta)		
Stefek et al. (2000) [89]	STZ	♂ Wistar rats			⇑		
Stobadine 0.05% w/w for 32 days					N		

Diabetogens alloxan (ALX) or streptozotocin (STZ), administered to Wistar or Sprague-Dawley (SD) rats, produced increases (⇑) or decreases (⇓) from normal levels of catalase activity as indicated in the top line for each study. Dose and route of diabetogen administration is indicated in the second line of column 2. Treatment with antioxidant chemicals produced no effect (○), further increase (⇑⇑), or normal activities (N) of catalase at the specified time, as indicated in the lower line(s) of each study.

[57] normalize renal activity, but no reversal of diabetic effects is seen with melatonin [58], aminoguanidine or desferrioxamine [68], or gemfibrozil [53]. Treatment with vitamin C, vitamin E, and β-carotene for 8 weeks elevates hepatic SOD activity in diabetic rats, which is normal without treatment [78].

Vitamins

Vitamins A, C, and E are diet-derived and detoxify free radicals directly. They also interact in recycling processes to generate reduced forms of the vitamins. α-Tocopherol is reconstituted when ascorbic acid recycles the tocopherol radical; dihydroascorbic acid, which is generated, is recycled by glutathione. These vitamins also foster toxicity by producing prooxidants under some conditions. Vitamin E, a component of the total peroxyl radical-trapping antioxidant system [91], reacts directly with peroxyl and superoxide radicals and singlet oxygen and protects membranes from lipid peroxidation. The deficiency of vitamin E is concurrent with increased peroxides and aldehydes in many tissues. There have been conflicting reports about vitamin E levels in diabetic animals and human subjects. Plasma and/or tissue levels of vitamin E are reported to be unaltered [92], increased [93], or decreased [60,94,95] by diabetes. Discrepancies among studies in terms of preventive or deleterious effects of vitamin E on diabetes-induced vascular aberrations may arise from the variety of examined blood vessels or the administered dose of vitamin E.

Nitrite Concentration

Increasing evidence suggests that oxidative stress and changes in nitric oxide formation or action play major roles in the onset of diabetic complications. Nitric oxide synthase oxidizes arginine to citrulline in the presence of biopterin, NADPH, and oxygen. Generally, nitric oxide at physiological levels produces many benefits to the vascular system. However, increased oxidative stress and subsequent activation of the transcription factor NF-κB have been linked to the development of late diabetic complications. NF-κB enhances nitric oxide production, which is believed to be a mediator of islet beta-cell damage. Nitric oxide may react with superoxide anion radical to form reactive peroxyl nitrite radicals.

A number of studies are continuing to examine the role of nitric oxide in diabetes mellitus. For example, subnormal hepatic nitric oxide concentrations in STZ-diabetic rats are restored after melatonin treatment to levels significantly higher than normal [58]. And, although elevated levels of nitric oxide levels in kidneys of 3 week diabetic rats are further enhanced by *S*-methyl-L-thiocitrulline treatment, administration of losartan along with *S*-L-thiocitrulline for 3–5 weeks normalizes the nitric oxide levels implying that angiotensin II is an important modulator of nitric oxide pathway in diabetes [96].

On the other hand, nitric oxide levels in plasma are decreased in alloxan-diabetic rats, an effect that can be abrogated by prior and simultaneous administration of L-arginine, a precursor of nitric oxide [67]. When *N*-monomethyl-L-arginine, a specific inhibitor of nitric oxide synthase, is given along with alloxan, the beneficial actions of L-arginine in diabetes are blocked. However, when sodium nitroprusside and L-arginine are administered simultaneously with alloxan for 5 days, nitric oxide production remains at control levels. These results suggest that both L-arginine and sodium nitroprusside, with the capacity to enhance nitric oxide levels in alloxan-diabetic animals, can prevent alloxan-induced islet beta-cell damage and the development of diabetes as well as restore the antioxidant status.

Finally, retinal nitric oxide levels are increased in alloxan-diabetes and experimental galactosemia in rats [97]. Aminoguanidine supplementation significantly inhibits retinal nitric oxide concentrations and normalizes the hyperglycemia-induced increases in retinal oxidative stress without lowering the blood hexose levels of these animals.

Nonenzymatic Glycosylated Proteins and Hyperglycemia

Diabetic hyperglycemia results in an increase in free-radical production by a mechanism involving glucose oxidation followed by protein glycation and oxidative degeneration [98]. Glycation (nonenzymatic glycosylation) involves the condensation of glucose with the ε-amino group of lysine, the α-amino group of an N-terminal amino acid or the amines of nucleic acids [99]. The first reaction is the formation of an unstable Schiff base, which reaches a steady state within hours [100] and is reversible. Rearrangement of the Schiff base into an Amadori product reaches a steady state in approximately 28 days and is also reversible. When molecules have slow turnover rates, these Amadori products undergo multiple dehydration reactions and rearrangements to irreversibly form AGEs [101]. AGEs are believed to be involved in the genesis of many of the irreversible complications of diabetes, including expanded extracellular matrix, cellular hypertrophy, hyperplasia, and vascular complications [102,103].

Markers used for estimating the degree of protein glycation in diabetes include fructosamine and glycated hemoglobin levels. Nonenzymatic glycation may also alter the structure and function of antioxidant

enzymes such that they are unable to detoxify free radicals, exacerbating oxidative stress in diabetes. For example, high glucose levels, leading to glycation and high levels of glycated proteins, modulate the activity of nitric oxide synthase directly or indirectly (through protein kinase C) [104].

Normoglycemia is a desired effect of any drug used either singly or in combination in the treatment of diabetes, but apart from insulin, only a limited number of drugs including melatonin, probucol, vitamins C and E plus β-carotene, and α-lipoic acid [51,52,63,77,78] reduce high blood glucose levels in diabetes. The majority of antioxidants do not reverse diabetes-induced hyperglycemia, and these agents must be given as adjuvants to insulin therapy.

Elevated glycosylated hemoglobin and fructosamine concentrations in diabetic Wistar rats are restored to normal levels after treatment with β-carotene (50 mg/kg) for a period of 40 days [105]. STZ-induced diabetic Sprague-Dawley rats demonstrate hyperglycemia, high levels of glycated hemoglobin A_{1c} and AGEs, as well as impaired acetylcholine-induced relaxations of the vascular segments. However, treatment with acarbose immediately after STZ, supplemented with low dose insulin (1 unit/day), restores both blood glucose and glycated hemoglobin A_{1c} to normal levels, but not the AGE content. Addition of 100 U/mL SOD normalizes the impaired vascular relaxation, suggesting an important role of superoxide radicals in diabetes-induced endothelial dysfunction [106].

Increased nonenzymatic glycation and AGEs are also postulated to contribute to cataract formation. Administration of aldose reductase inhibitors (0.06% tolrestat or polnalrestat, 0.0125% AL-1576 for 8 weeks) in the diet of STZ-induced diabetic rats results in reduced sorbitol levels, inhibition of cataract formation, lowered concentrations of glycosylated lens proteins, and slightly reduced lenticular AGE levels compared to untreated diabetic rats after 45 and 87 days of diabetes [107].

Treatment of diabetes in male CF1 mice with acetylsalicylic acid (0.16% w/w in diet starting 30 min after STZ injection) blocks the accumulation of lipoperoxide aldehydes, reduces hyperglycemia, and prevents the inactivation of heme enzymes, δ-aminolevulinic dehydrase, and porphobilinogen deaminase [74]. This inhibition of protein glycosylation through acetylation of free amino groups and lowering of blood glucose by acetylsalicylic acid may prevent some of the complications of diabetes.

STZ-diabetes induces a 10-fold increase in γ-glutamyl transferase activity in rat liver [108–110], resulting in decreased biliary excretion of glutathione and other chemicals [111]. Although regulation of γ-glutamyl transferase activity has been shown to be independent of message or expression [108], alterations in kinetic and other physical characteristics of the enzyme in diabetic rats implicate glycation as a mechanism of regulation [112]. A decrease in glutathione excretion into bile in diabetics may have important consequences such as impairing the capacity of the intestine to detoxify dietary lipid peroxides or carcinogens. On the other hand, increased reclamation of glutathione may benefit the liver by increasing its ability to detoxify reactive prooxidants within the liver.

CONCLUSIONS

STZ- or alloxan-induced diabetes in rats represent well-established animal models of type 1 insulin-dependent, diabetes mellitus. Increased production of high levels of oxygen free radicals has been linked to glucose oxidation and nonenzymatic glycation of proteins which contribute to the development of diabetic complications. Protective effects of exogenously administered antioxidants have been extensively studied in animal models within recent years, thus providing some insight into the relationship between free radicals, diabetes, and its complications. In vitro and clinical studies may provide additional useful ways to probe the interconnections of oxidant stress and diabetes, and there is a need to continue to explore the mechanisms by which increased oxidative stress accelerates the development of complications in diabetes.

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