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Ascorbic Acid Repletion: A Possible Therapy for Diabetic Macular Edema?

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Abstract

Macular edema poses a significant risk for visual loss in persons with diabetic retinopathy. It occurs when plasma constituents and fluid leak out of damaged retinal microvasculature in the area of the macula, causing loss of central vision. Apoptotic loss of pericytes surrounding capillaries is perhaps the earliest feature of diabetic vascular damage in the macula, which is also associated with dysfunction of the endothelium and loss of the otherwise very tight endothelial permeability barrier. Increased oxidative stress is a key feature of damage to both cell types, mediated by excess superoxide from glucose-induced increases in mitochondrial metabolism, as well as by activation of the receptor for advanced glycation end products (RAGE). The latter in turn activates multiple pathways, some of which lead to increased oxidative stress, such as those involving NF- κ B, NADPH oxidase, and endothelial nitric oxide synthase. Such cellular oxidative stress is associated with low cellular and plasma ascorbic acid levels in many subjects with diabetes in poor glycemic control. Whether repletion of low ascorbate in retinal endothelium and pericytes might help to prevent diabetic macular edema is unknown. However, cell culture studies show that the vitamin prevents high-glucose and RAGE-induced apoptosis in both cell types, that it preserves nitric oxide generated by endothelial cells, and that it tightens the leaky endothelial permeability barrier. Although these findings need to be confirmed in pre-clinical animal studies, it is worth considering clinical trials to determine whether adequate ascorbate repletion is possible and whether it might help to delay or even reverse early diabetic macular edema.

Keywords

ascorbate; diabetes; macular edema; pericytes; endothelial cells

Clinical Impact of Diabetic Retinopathy with Macular Edema

Diabetic retinopathy is one of the most feared complications of the disease. Over the period of 2005-2008, retinopathy was present in almost 30% of people over the age of 40 with diabetes, causing severe vision loss or blindness in 4.4 % [1]. In patients with retinopathy,

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early changes known as background retinopathy manifest as capillary microaneurysms, exudates, and venous changes that may progress to vessel loss and ischemia. This in turn causes new vessel growth or proliferation into the vitreous humor. These fragile vessels are prone to hemorrhage and eventual scarring, causing both decreased vision and in some cases, retinal detachment. Associated with the development of retinopathy is leakage of fluid and vascular components into the retinal substance causing swelling and eventually hard exudates composed of serum lipids and proteins. When this leakage occurs in the macula or region of greatest visual acuity, it can cause significant loss of vision. Indeed, macular edema is perhaps the most frequent cause of moderate to severe visual loss [2, 3]. This is especially true in Type 2 diabetes mellitus (T2DM), where 25% of patients taking insulin develop clinically significant macular edema over 10 years and which is worse in patients with poor glycemic control [4].

Treatment of diabetic macular edema has evolved rapidly over the last decade. As shown by the Diabetes Control and Complications Trial and its follow-up EDIC Study in the 1990's, tight glycemic control makes a difference in onset and progression of retinopathy and macular edema in type 1 diabetes mellitus (T1DM) [5]. The UKPDS trial confirmed this for retinopathy progression in T2DM [6]. However, tight glycemic control is not easy to achieve in most patients, despite compliance with medications and diet, thus allowing retinopathy progression. Although laser therapy in proliferative retinopathy is clearly beneficial, it has been superseded for macular edema by pharmacotherapy with intravitreal injection of anti-Vascular Endothelial Growth Factor (VEGF) antibodies [7] and in poor responders by vitreal steroid implants [8]. Other therapies of promise, such as fibrinolytics [9, 10] and inhibition of protein kinase C with ruboxystaurin [11] have not moved into common use. Indeed, ruboxystaurin has been removed from consideration by the manufacturer. Thus, there remains a major unmet need for non-toxic therapies that might delay or prevent early macular edema before, or that might serve as an adjunct to more invasive therapies.

Pathogenesis of early diabetic macular edema

In devising additional therapies for diabetic macular edema, it is necessary to consider early phases of its pathogenesis. The hallmark of early diabetic macular edema is dysfunction of the microvasculature. Loss of pericytes is one of the earliest changes in diabetic retinopathy [12-14]. Pericytes surround the capillary basement membrane, forming a sheath of dendritic-like processes that enclose small blood vessels (Fig. 1). Pericyte density per endothelial cell is more than two-fold higher in the retina than in the cerebral cortex and other organs in rodents [15]. This increased pericyte density suggests a critical role for these cells in the retina. Pericytes function to provide structural support of small vessels [16], enable angiogenesis [17], regulate blood flow [18], and tighten the endothelial permeability barrier [19]. Disruption of the latter results in leakage of fluid and serum components into the interstitium, thus causing macular edema.

Pericyte "dropout" in diabetic retinopathy is due largely to apoptosis. This is evident both in primary cultures of retinal pericytes [20-22] and *in vivo* [13, 23]. With loss of pericytes, the underlying endothelial cells become dysfunctional [24], lose their ability to generate nitric oxide [25, 26], and can no longer maintain an adequate permeability barrier [27, 28]. High

glucose concentrations also cause loss of endothelial cells due to apoptosis [29, 30], leaving regions of acellular capillaries that are prone to aneurysm formation and further leakage [13, 14, 31]. If this leakage occurs in the center of the macula, or foveal region, vision can be severely compromised.

Glucose-induced oxidative stress damages pericytes and endothelial cells

Oxidative stress is a key factor implicated in damage to both pericytes and endothelial cells in early diabetic retinopathy. This is due to excess superoxide generation, both as a by-product of increased glucose metabolism and in response to cell binding of Advanced Glycation End-products (AGE's) [32]. Regarding the former, obligate cellular uptake of glucose at high concentrations on facilitative glucose transporters increases mitochondrial respiration with release of superoxide as a byproduct [33, 34] (Fig. 2). AGE's cause oxidative stress by more complex mechanisms. They are generated with time as glucose non-enzymatically binds to lysines of various proteins. Subsequent reactions lead to adjuncts that both decrease function of the involved proteins and convert them to ligands for the Receptor for AGE's (RAGE). Numerous ligands have been implicated in activation of endothelial and pericyte RAGE. For example, in pericytes these include glycosylated serum proteins such as albumin [20, 21, 35] and glycolysis-derived methylglyoxal [36]. RAGE is also bound by ligands produced by damaged leukocytes (e.g., high mobility group box 1 protein (HMGB1), calgranulins) that both increase its activation and expression in endothelial cells [37-39]. RAGE in turn activates multiple intracellular transduction pathways, some of which lead to increased superoxide generation [37, 40] (Fig. 2).

RAGE activation increases damaging reactive oxygen and nitrogen species (ROS/RNS) in both pericytes [20, 21] and endothelial cells [32, 40]. That these ROS/RNS are damaging is suggested by studies in which a variety of antioxidants prevented pericyte toxicity and death due to AGE's [21, 35, 41]. Similarly, in human umbilical vein endothelial cells (HUVECs) several antioxidants prevented RAGE-induced leaks in endothelial barrier permeability [42]. There are at least three pathways by which RAGE activation increases superoxide and downstream ROS/RNS in pericytes and endothelial cells (Fig. 2).

First, RAGE activates NADPH oxidase (NOX) in both cell types. In bovine retinal pericytes, 48 h of high glucose more than doubled NOX expression and activity [43]. Further, inhibition of NOX in pericytes decreased both high glucose- [43] and AGE-induced apoptosis [41]. Similarly, in cultured human endothelial cells RAGE activation generated ROS/RNS (Fig. 2), an effect blocked by inhibition of NOX [32, 38]. In *ex vivo* studies of mouse retinas, capillary permeability increased with perfusion of AGE-modified bovine serum albumin, an effect again blocked by inhibition of NOX and by scavenging free radicals [39]. Additional inhibitor experiments in that study suggested that NOX activation by RAGE required activity of a calcium-dependent protein kinase C, presumably activated by calcium released due to RAGE up-regulation of phospholipase c [44].

Second, the proinflammatory NF- κ B pathway is also activated by RAGE in both retinal pericytes [45, 46] and endothelial cells [44, 47] (Fig. 2). In retinas from human diabetics, nuclear NF- κ B immunostaining was increased in pericytes compared to control eyes,

although it was not increased in endothelial cells [22]. On the other hand, sustained NF- κ B expression was observed in endothelial cells from vessels of diabetic rats [48], thus supporting the notion that it is increased in both cell types in diabetes. With regard to mechanism, treatment of cultured rat retinal pericytes with methylglyoxal caused apoptosis that was prevented by inhibition of NF- κ B or inducible nitric oxide synthase (iNOS) [36]. In that same study, pericyte apoptosis was increased in rat eyes injected with methylglyoxal, as were NF- κ B and iNOS. Finally, inhibition of NF- κ B also decreased iNOS expression in pericytes, implicating nitric oxide (NO) or peroxynitrite in the apoptotic process. That excess ROS/RNS from iNOS activation may be involved in diabetic retinal disease is also supported by the finding that 12 month-old diabetic C57Bl/6 mice lacking iNOS had decreased numbers of acellular retinal capillaries and pericyte ghosts compared to wild-type diabetic controls [49]. However, a 24 h exposure of bovine retinal pericytes to 30 mM glucose decreased both iNOS expression and function [50]. Thus although short-term exposure to high glucose may not induce pericyte iNOS, this does appear to occur with longer exposure and constitutes a third potential pathway to generate ROS/RNS, at least in pericytes.

Third, in endothelial cells, endothelial nitric oxide synthase (eNOS) may also produce ROS/RNS (Fig. 2). NO that is normally generated by eNOS can react very rapidly with superoxide derived from NOX and mitochondrial sources during high glucose and RAGE activation to form peroxynitrite [51]. This serves to both deplete NO and to increase potentially damaging protein oxidation and nitration. Superoxide-derived ROS can also oxidize tetrahydrobiopterin, the required co-factor for endothelial nitric oxide synthase (eNOS). This uncouples eNOS, which then yields superoxide instead of NO. Thus, eNOS becomes a source for more superoxide [52]. However, uncoupling of eNOS was not observed in AGE-BSA stimulated retinal permeability; rather inhibition of eNOS with N^ω-nitro-L-arginine methyl ester (L-NAME) enhanced barrier leakage during RAGE activation [39]. This favors the notion that NO might scavenge superoxide generated by RAGE activation, albeit with the generation of peroxynitrite. Other defense mechanisms may also come into play, such as up-regulation of protective enzymes (e.g., superoxide dismutase, glyoxalase I [53]). Nonetheless, increased oxidative stress will deplete endogenous cytosolic antioxidants such as ascorbate and GSH [54], leading to protein, DNA, and lipid oxidation.

Role of antioxidants in the therapy of macular edema in diabetic retinopathy

Macular degeneration is another disease of the macula that causes significant vision loss in older people. It is thought to be caused, at least part, by increased oxidative stress in the macular region. Clinical evidence for this derives from the Age-Related Eye Disease Study (AREDS), which showed that high dose zinc and vitamins C and E decreased age-related macular degeneration by about 25% over 6 years [55]. Kowluru and colleagues applied this finding to retinopathy in rats made diabetic with streptozotocin. They found that supplements of the AREDS antioxidants, including ascorbate, decreased numbers of acellular capillaries by as much as 50% in rats with 12 months of diabetes compared to a control group of diabetic animals [31, 56]. This was associated with decreases in several

markers of oxidative stress, including nitrotyrosine and oxidized DNA [31]. Whereas these results support ascorbate as an antioxidant that might be useful in treating macular edema of diabetic retinopathy, clinical trial data are lacking [57].

Vitamin C depletion as a marker for oxidative stress in diabetes

Ascorbic acid is one of the best cellular indicators of oxidative stress [58, 59]. Indeed, persons with diabetes complications and thus increased oxidative stress have long been known to have plasma ascorbate concentrations as low as 40% of normal [60-63]. This deficit occurs even though these subjects have presumably adequate intakes of the vitamin (~150 mg/d) [60]. It was initially thought that poor glycemic control caused the low plasma ascorbate concentrations due to loss of ascorbate in the urine from glucose-induced osmotic diuresis [64]. However, this hypothesis was not supported by a study of ascorbate-deficient diabetics in which poor glycemic control decreased rather than increased urinary loss of the vitamin [65]. Further, despite reasonable glycemic control and adequate intakes, many studies still show decreased plasma ascorbate concentrations in T2DM [60, 65-67]. Although there is concern that ascorbate assays in early studies of diabetes suffered from methodologic problems [68], more recent studies in the last decade using HPLC-based ascorbate assays have confirmed low plasma ascorbate levels in T2DM subjects [66, 69]. Further evidence that this decrease is real and reflective of total body stores is shown by diminished leukocyte ascorbate concentrations in both T1DM [70] and T2DM [71]. Hyperglycemia has also been shown to decrease erythrocyte ascorbate concentrations, an effect correlated with decreased erythrocyte deformability and possible microvascular angiopathy [72].

T2DM subjects with diabetic microvascular disease and retinopathy in particular have lower plasma ascorbate concentrations than T2DM subjects without these complications [61, 62, 73]. This difference in ascorbate levels was evident even within the first year of T2DM [74]. Perhaps most relevant to diabetic retinopathy, despite fair glycemic control (hemoglobin A_{1C} = 8.1%), T1DM patients with proliferative retinopathy had retinal **vitreous** ascorbate concentrations only 23% of normal [75]. Plasma ascorbate levels were also decreased, but only to 43% of the levels in control subjects. This differential suggests that the retina in patients with diabetic retinopathy may also be under significant oxidative stress.

In further support of ascorbate as a marker for oxidative stress in diabetes, plasma ascorbate was decreased in elderly T2DM subjects when other markers of oxidative stress were unaffected [61]. Second, ascorbate may be difficult to fully replete in T2DM patients. In one study an oral dose of 1 g daily increased plasma ascorbate in 20 T2DM subjects with complications 3-fold from 42 to 127 μ M at three weeks, but at 6 weeks of therapy the level had decreased significantly to 87 μ M [73]. In another study of T2DM, oral ascorbate supplements of 800 mg/d doubled plasma ascorbate from 23 to 48 μ M at 4 weeks, but this was less than expected in non-diabetic subjects [67]. These results suggest that the oxidative stress of diabetes causes a high turnover of ascorbate.

Ascorbate functions that may decrease pericyte loss and endothelial dysfunction in retinopathy

Ascorbate may not simply be a marker for increased oxidative stress in diabetes, but its systemic or even local deficiency may promote pericyte and endothelial dysfunction in diabetic retinas. As noted above, ascorbate is well established as a key antioxidant in both plasma and in cells, where it both scavenges radical species and recycles several crucial cellular molecules [76]. Ascorbate serves as a primary antioxidant by detoxifying exogenous radicals or superoxide generated by mitochondrial metabolism, NOX, or uncoupled eNOS. At the low mM concentrations of ascorbate likely to exist in endothelial cells [77, 78] and pericytes [79], ascorbate effectively scavenges superoxide [80, 81] and peroxynitrite [82, 83] (Fig. 3). These damaging molecules are increased in endothelial cells [84] and pericytes [20] cultured at high glucose and by hyperglycemia *in vivo* [85].

Ascorbate also recycles and thus preserves several readily oxidized molecules in the cell. It repairs both protein [86] and lipid radicals [87]. For example, when α -tocopherol reacts with lipid peroxyl radicals in the lipid phase, it is oxidized to the α -tocopheroxyl radical, which ascorbate reduces to α -tocopherol [88]. The resulting ascorbate radical is then rapidly recycled back to ascorbate by NAD(P)H-dependent reductases [89]. The ascorbate radical may also dismutate, producing one molecule of ascorbate and another of dehydroascorbate, the two-electron oxidized form of ascorbate. The latter can be reduced back to ascorbate by cytosolic NADPH-dependent dehydrogenases and thiol transferases [90]. Both forms of ascorbate can also be taken up by mitochondria on the SVCT2 (ascorbate) [91, 92] and GLUT10 (dehydroascorbate) [93], where ascorbate can directly scavenge excess superoxide and dehydroascorbate can do so after reduction back to ascorbate. However, low plasma ascorbate concentrations and inadequate recapture of dehydroascorbate due to inhibition of its uptake by glucose on GLUT-type glucose transporters may result in low mitochondrial ascorbate concentrations.

A key recycling function of ascorbate is to preserve nitric oxide (NO), albeit indirectly. In addition to scavenging superoxide that will otherwise react with NO to form peroxynitrite [94], ascorbate sustains eNOS activity by recycling tetrahydrobiopterin [83, 95]. Ascorbate does this by a one-electron reduction of the trihydrobiopterin radical that is generated in the effective eNOS enzyme cycle, thus reactivating the enzyme and preventing its uncoupling [83]. This reaction is specific for ascorbate, since thiols are ineffective [83].

Another non-antioxidant function of ascorbate is to sustain the activity of various dioxygenase enzymes [96]. These enzymes use α -ketoglutarate and molecular oxygen to hydroxylate their respective substrates. They have an active site non-heme iron that is kept in its functional, reduced form by ascorbate. Best known among the prolyl hydroxylases are those that hydroxylate proline and lysine in procollagen, thus allowing proper folding of the chain and release from the cell as mature collagen. This function of course allows both endothelial cells and pericytes to lay down type IV collagen in the vessel wall. Another prolyl hydroxylase isoform that requires ascorbate is the enzyme that hydroxylates HIF-1 α . Hydroxylation of HIF-1 α on specific prolines or lysines targets it for ubiquitination and proteosomal destruction [97]. Ascorbate facilitates this process and thereby lowers HIF-1 α .

levels [98, 99] (Fig. 3). Lack of ascorbate prevents this hydroxylation and allows HIF-1 α to accumulate. The surviving HIF-1 α then forms a nuclear transcription complex that activates diverse pathways, including those for glucose metabolism, angiogenesis, and inflammation [100] (Fig. 2). Indeed, high glucose is one of only a few non-hypoxic factors that can increase HIF-1 α mRNA/protein in various cell types [101], including brain endothelial cells [102]. The mechanism by which high glucose induces HIF-1 α probably relates to RAGE activation [103] (Fig. 2). In turn, high glucose-induced increases in HIF-1 α also increase endothelial barrier permeability, perhaps by increasing VEGF [102]. However, the cells in that study were ascorbate-deficient; lack of ascorbate could thus have caused increased HIF-1 α and subsequent loss of barrier function.

Ascorbate has been shown to decrease the NF- κ B pathway in a variety of cultured cells, including HUVECs stimulated by tumor necrosis factor- α (TNF- α) [104] (Fig. 3). This was associated with decreased phosphorylation of I κ B, which prevented NF- κ B translocation to the nucleus. It was not mediated by p38 MAP kinase, but rather due to inhibition of NF- κ B inducing kinase and IKK β kinases. How ascorbate decreased I κ B phosphorylation was not determined. It is known that RAGE activation increases NF- κ B in HUVECs [47], but whether ascorbate inhibits NF- κ B in response to high glucose/RAGE is unknown.

Lastly, studies from our laboratory have shown that ascorbate acutely tightens the endothelial permeability barrier. This effect occurs over 90 minutes and is not due to longer term increases in collagen matrix deposition [105], which had previously been observed for ascorbate in HUVECs [106]. The mechanism by which ascorbate decreases basal endothelial barrier permeability appears to require NO, since it was prevented by inhibition of either eNOS or downstream guanylate cyclase [107]. Increases in ascorbate to what are likely physiologic intracellular concentrations (1-2 mM) in HUVECs also prevented/reversed increases in endothelial barrier permeability caused by known oxidative stressors, including H₂O₂ [108], oxidized low density lipoprotein [109], and intracellular ferric iron [110]. Generation of oxidative stress may also contribute to the increase in barrier permeability seen with a 90 minute treatment of HUVECs with VEGF, since the growth factor both increased ROS in HUVECs and depleted cellular ascorbate [108]. In that study, blockade of eNOS with L-NAME (but not DNAME) as well as increasing tetrahydrobiopterin by treating the cells with its precursor sepiapterin prevented the barrier leakage due to VEGF. This suggests that uncoupling of eNOS with superoxide production might have contributed to the VEGF-induced barrier leak. It also fits with the notion that ascorbate preservation of NO might be involved in its ability to block barrier leak due to VEGF. Whatever the mechanism, the ability of ascorbate to block VEGF-induced increases in endothelial barrier permeability suggests that repletion of its deficiency in diabetic eyes with proliferative retinopathy [75] and perhaps macular edema could be a therapeutic adjunct to intravitreal injection of anti-VEGF antibodies.

Ascorbate loading of HUVECs also completely prevented the endothelial barrier leak caused by short-term treatment with the inflammatory agent thrombin [111]. In that study, ascorbate acting through NO increased cyclic GMP and prevented the thrombin-induced decrease in cyclic AMP. The latter was associated with activation of Epac1/Rap1 as well as Rac1, leading to maintenance of cortical actin, thus counteracting a decrease in this pathway due to

thrombin. A second effect of cyclic AMP preservation was partial inhibition of the thrombin-induced increase in RhoA and subsequent phosphorylation of myosin light chain, thus decreasing stress fiber formation.

Potential roles for ascorbate in prevention of diabetic macular edema

Ascorbate depletion could impact two key aspects involved in the development of diabetic macular edema. First, it could prevent loss of pericytes due to apoptosis. As noted above, pericyte apoptosis is an early and possibly initiating feature of vascular leak in diabetes. We found that treatment of microvascular brain and retinal pericytes with physiologic plasma concentrations of ascorbate (50-100 μ M) prevented apoptosis during culture at both 5 mM and 25 mM glucose [112]. Apoptosis during culture at high glucose was due largely to RAGE activation, since it was prevented by sub-micromolar concentrations of a potent RAGE antagonist. A 24 hour treatment of pericytes with the RAGE agonists AGE-coupled bovine serum albumin and HMGB1 increased apoptosis, an effect blocked by ascorbate, but not by other antioxidants. Ascorbate also prevented apoptosis in endothelial cells [113] and supplementation of both ascorbate and α -tocopherol prevented retinal pericyte/endothelial cell apoptosis *in vivo* in diabetic rats [114].

Second, ascorbate could prevent endothelial dysfunction due to high glucose and RAGE activation. Two aspects of endothelial dysfunction are especially relevant to diabetes. As discussed earlier, ascorbate preserves NO. This should allow more NO to reach smooth muscle and thus should improve vascular reactivity in diabetes. This may contribute to its ability to improve acetylcholine-induced (i.e., NO-dependent) vasodilation in persons with either acute hyperglycemia [115] or diabetes [116].

Diabetic hyperglycemia also increases endothelial barrier permeability [117, 118], which is considered a precursor for diabetes microvascular complications [27]. Ascorbate tightens the leaky endothelial permeability barrier caused by high glucose [42, 119] and RAGE agonists [42]. This barrier tightening by ascorbate occurs in cells treated for only 90 minutes with physiologic plasma concentrations of ascorbate (25-100 μ M). Since ascorbate decreases barrier leakage in cells that have been cultured for 5-6 days with high glucose (25 mM) or for 24 hours with RAGE agonists, it is reversal rather than prevention. Improved vascular reactivity and a tighter endothelial permeability barrier could thus be important functions of ascorbate related to diabetic macular edema.

However, there are caveats to consider as to whether this translates to clinical benefit. First, although high dose supplements of multiple antioxidants, including ascorbate, decrease rodent diabetic retinal apoptosis *in vivo* and *in vitro* [31, 56], this does not mean that more modest doses will do so in humans. Second, assimilation of ascorbate in mammals and humans is very efficient, but saturation of intestinal uptake and renal loss limits levels that can be achieved *in vivo* [120]. Failure to achieve adequate cellular ascorbate concentrations with moderate oral dose ascorbate repletion may thus have accounted for failure to enhance NO-dependent vascular relaxation in T2DM subjects [67]. Third, most studies in cultured cells described above have compared effects at very low to moderately high levels of cellular ascorbate. Since treating cells with low physiologic concentrations of ascorbate prevented

apoptosis (pericytes) and barrier leakage (endothelial cells), one could ask whether additional ascorbate would have an effect. This might explain why oral ascorbate supplements did not enhance vascular reactivity compared to placebo in the studies of Chen, et al., since that reactivity was already robust.

Finally, with extensive vascular damage such as might be present in diabetes, there could be release of ferric iron or other transition metals, recycling of which by ascorbate might have a pro-oxidant or deleterious effect. Ascorbate can act as a pro-oxidant in the test tube and at high concentrations around some cancer cells [121, 122]. This pro-oxidant effect is due to generation of H₂O₂ in a Fenton type reaction. Indeed, care must be taken in cell culture studies to rule-out the possibility that the effects of added ascorbate are really due to generation of H₂O₂ in the culture medium. Nevertheless, the general consensus is that ascorbate does not have deleterious pro-oxidant actions in normal physiology [76]. While these are valid concerns regarding possible pro-oxidant effects of the vitamin, ascorbate treatment in many diabetics would be repletion of a deficit, not supplementation. This deficit appears to be as much as 80% in the vitreous fluid of T1DM subjects with proliferative retinopathy [75].

Given the need for additional therapies to slow diabetic retinopathy and macular edema progression, the demonstrated deficiency of the vitamin in diabetes with poor glycemic control, the encouraging *in vitro* and pre-clinical findings described above, the expected safety of the vitamin in doses as high as 2 grams daily, it certainly is reasonable to consider well-designed clinical trials of vitamin C repletion in diabetics with macular edema.

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Abbreviations

AGE	advanced glycation end-products
eNOS	endothelial nitric oxide synthase
HMGB1	high mobility group box 1 protein
HUVECs	human umbilical vein endothelial cells
iNOS	inducible nitric oxide synthase
NOX	NADPH oxidase
L-NAME	(N ^ω -nitro-L-arginine methyl ester)
NO	nitric oxide
RAGE	receptor for advanced glycation end-products
RNS	reactive nitrogen species
ROS	reactive oxygen species
T1DM	type 1 diabetes mellitus

T2DM	type 2 diabetes mellitus
VEGF	vascular endothelial growth factor

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Highlights

- Macular edema is a major cause of vision loss in poorly controlled diabetes
- Macular edema is caused by breakdown of the endothelial permeability barrier due to pericyte loss and endothelial dysfunction
- Oxidative stress due to excess superoxide is a major contributor to barrier leak
- Ascorbic acid is depleted by oxidative stress in persons with diabetes in poor glycemic control
- Repletion of ascorbate could decrease vascular leak in diabetes by preventing apoptotic cell death and by tightening the endothelial permeability barrier

Figure 1.
Pericyte and endothelial anatomy.

Figure 2.

Sources of oxidative stress induced by RAGE activation in endothelial cells. RAGE activation enhances the synthesis and translocation of NF-κB by multiple mechanisms. It also activates NOX to generate superoxide ($O_2^{\bullet-}$), which further activates NF-κB via RAS and the phosphoinositide 3-kinase and Akt pathways. Superoxide is also generated as a by-product of increase mitochondrial metabolism due to high intracellular glucose concentrations. Downstream products of superoxide damage cellular proteins, lipids, and DNA. For example, reaction of superoxide with NO generated by eNOS forms peroxynitrite, which is a powerful oxidizing and nitrating agent. Abbreviations not defined in the text: Akt, protein kinase B; EPO, erythropoietin; ERK1/2, extracellular-regulated-kinase1/2; IL, interleukin; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; PDGF-β, platelet-derived growth factor- β; PKC, protein kinase C; rac1, Ras-related C3 botulinum toxin substrate 1; TGF- β, transforming growth factor- α; TNF- α; tumor necrosis factor- α.

Figure 3.

Mechanisms by which ascorbate (AA) decreases oxidative stress in endothelial cells. Ascorbate enters endothelial cells and pericytes on the SVCT2 (Sodium-dependent Vitamin C Transporter-2) and likely achieves low millimolar levels in both cell types. At this concentration, it preserves NO by recycling tetrahydrobiopterin in eNOS, as well as by reducing superoxide to H_2O_2 , which is then destroyed by catalase. Removal of superoxide decreases RAS-mediated activation of NF- κ B, as well as formation of peroxynitrite, which is also scavenged by ascorbate. Ascorbate also decreases translocation of NF- κ B to the nucleus and inactivates HIF-1 α .