Abstract: In studies of vitamin E effectiveness in diabetes, there are still controversies surrounding negative observational and positive experimental results. However, there is no controversy that antioxidant vitamin E is regenerated from its pro-oxidant tocopheroxyl radical by a network of interacting co-antioxidants. The network of interacting co-antioxidants has only been studied individually. The hypothesis we propose is that a vitamin E regeneration system (VERS) model based on the complex interactions of the co-antioxidants provides a rationale for vitamin E supplementation as a therapeutic adjunct in diabetes. Furthermore, the factors considered prior to the use of Vitamin E as a supplement in diabetes research and therapy, the effectiveness of vitamin E supplementation and the limitations have been identified in the literature. There is no single study of vitamin E supplementation or efficacy that has determined vitamin E levels in combination with all of the co-antioxidants that interact to regenerate oxidised vitamin E. Therefore, there is a lack of good evidence for or against vitamin E being unilaterally depleted in the antioxidant network. There is also lack of rationale for choice of co-antioxidant supplementation. In essence, the normal conditions for effective antioxidant activity of vitamin E supplementation have yet to be fully explored. We propose a coherent model of VERS, and recommend that VERS status needs to be assessed, as part of evidence-based clinical practice to determine whether vitamin E should be recommended for the diabetic patient. We also propose an algorithm, based on the antioxidant activity and confounding factors, to guide the formulation of a credible hypothesis for clinical trials in assessing the function of vitamin E and treatment outcomes. The proposed model hinges on pertinent questions that have to be addressed to avoid organising a clinical trial that has been identified as biased.

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The ‘vitamin E regeneration system’ (VERS) and an algorithm to justify antioxidant supplementation in diabetes – a hypothesis

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Abstract

In studies of vitamin E effectiveness in diabetes, there are still controversies surrounding negative observational and positive experimental results. However, there is no controversy that antioxidant vitamin E is regenerated from its pro-oxidant tocopheroxyl radical by a network of interacting co-antioxidants. The network of interacting co-antioxidants has only been studied individually. The hypothesis we propose is that a vitamin E regeneration system (VERS) model based on the complex interactions of the co-antioxidants provides a rationale for vitamin E supplementation as a therapeutic adjunct in diabetes. Furthermore, the factors considered prior to the use of Vitamin E as a supplement in diabetes research and therapy, the effectiveness of vitamin E supplementation and the limitations have been identified in the literature. There is no single study of vitamin E supplementation or efficacy that has determined vitamin E levels in combination with all of the co-antioxidants that interact to regenerate oxidised vitamin E. Therefore, there is a lack of good evidence for or against vitamin E being unilaterally depleted in the antioxidant network. There is also lack of rationale for choice of co-antioxidant supplementation. In essence, the normal conditions for effective
antioxidant activity of vitamin E supplementation have yet to be fully explored. We propose a coherent model of VERS, and recommend that VERS status needs to be assessed, as part of evidence-based clinical practice to determine whether vitamin E should be recommended for the diabetic patient. We also propose an algorithm, based on the antioxidant activity and confounding factors, to guide the formulation of a credible hypothesis for clinical trials in assessing the function of vitamin E and treatment outcomes. The proposed model hinges on pertinent questions that have to be addressed to avoid organising a clinical trial that has been identified as biased.
Background to hypothesis

There is oxidative stress (OS) involvement in diabetes mellitus (DM).\(^{(1;2)}\) The role of antioxidants, including nutritional supplements, is not disputed. The perceived problem with antioxidant therapy in DM is the notion that the toxic effect of hyperglycaemia is stopped if vitamins C and E are recommended at the onset of disease progression.\(^{(3)}\) However, the controversy surrounding antioxidant vitamins has recently been hallmarked by the publication of the Guidelines of the American Heart Association, which has listed antioxidant vitamin supplements among therapies not recommended for CVD prevention, because it is not useful and may cause harm.\(^{(4)}\) This raises the need for both clinicians and researchers to rethink the mechanisms of antioxidant activity of vitamin E, how the widely reported toxicity arises and how the toxicity can be avoided, if OS and its consequential oxidative damage is a factor to consider in the dietary or nutraceutical management of diabetes. We present an overview of antioxidant vitamin E activity in diabetes and propose a ‘vitamin E regeneration system’ (VERS) model, which explains how toxicity is avoided in normal physiology.

The antioxidant effect of vitamin E in diabetes: Cardiovascular complications of diabetes arise through a number of pathways including polyol, hexosamine, protein kinase C, and glycation pathways. The unifying occurrence in these pathways is the overproduction of the oxidant superoxide ion (\(O_2^-\)) that increases susceptibility to intracellular OS.\(^{(2)}\) Specifically, there is erythrocyte oxidative stress (EOS) in DM, which can arise from the hyperglycaemia-induced production of free radicals.\(^{(2;5)}\) Altered erythrocyte activity and changes in vascular events that culminate in macrovascular complications of DM accompany EOS.\(^{(6)}\)
Diabetes mellitus/Hyperglycaemic status

\[ \text{Hyperglycaemia-induced superoxide production} \]

\[ \text{O}_2^- \text{ (superoxide radicals)} \]

Dismutation – spontaneous or catalyzed by SOD

\[ \text{H}_2\text{O}_2 \]

\[ \text{Frontline point of antioxidation by GSH – catalyzed by Gpx-1} \]

\[ \text{Fenton reaction* – driven by oxidant transition metal ions, Fe}^{2+} \text{ or Cu}^{+} \]

\[ \text{HO}^\cdot (\times 2 \text{ hydroxyl radicals}) \]

2Lipid-OH

\[ \text{Cellular membrane lipid peroxidation} \]

2H\text{O}

\[ \text{Lipid-O}^\cdot (\times 2 \text{ lipid peroxyl radicals}) \]

\[ \text{Exponential chain reactions} \]

\[ \text{Point of antioxidation by both vitamin E and coenzyme Q}_{10} \]

Oxidative stress

\[ \text{Via altered erythrocyte membrane fluidity and its sequelae} \]

Vascular disease complications

Fig. 1: Schematic sequence of how the major reactive oxygen species cause oxidative stress in the erythrocyte. *In the erythrocyte, oxy-haemoglobin readily enhances the process, which manifests as methaemoglobininaemia being indicated by metHb level.\(^{(4, 5, 7)}\)

Figure 1 is a simple illustration of three phases in the process of generation of reactive oxygen species (ROS: \( \text{O}_2^- \), \( \text{H}_2\text{O}_2 \) and \( \text{OH}^\cdot \)) through to vascular pathology. The first phase of OS is initiated by the generation of ROS, which depletes ‘reduced glutathione
(GSH) levels. The second phase involves free radical reactions, including lipid peroxidation, which is marked by high levels of malondialdehyde. It is at this phase that vitamin E is necessary to quench lipid peroxidation. The third phase is oxidative damage that occurs due to progressive OS, and low levels of reduced vitamin E. It shows that lipid peroxidation is an ultimate step of OS, preceded by biochemical processes leading to the formation of molecules, which can be important as targets for other antioxidant therapies different from vitamin E.\(^{(7)}\) It also illustrates that vascular damage is a phase in the disease progression that is beyond the range of vitamin E activity. That is, although vitamin E can prevent the occurrence of vascular damage, any damage that occurs is beyond the stage where vitamin E acts.

The antioxidation reaction of Vitamin E (Tocopherol-OH) involves one-electron reduction at the lipid phase via donation of a hydrogen atom (H') to a lipid peroxy radical to form the lipid peroxide (LOOH, see EQ 1).\(^{(8)}\) The lipid peroxide can thereafter be deactivated by GSH in the aqueous phase (EQ2).

\[
\text{LOO}^+ + \text{Tocopherol-OH} \rightarrow \text{LOOH} + \text{Tocopherol-O}^\cdot \quad \text{EQ 1}
\]

\[
\text{LOOH} + 2\text{GSH} \rightarrow \text{LOH} + \text{GSSG} + \text{H}_2\text{O} \quad \text{EQ2}
\]

In the absence of vitamin E, lipid peroxy radicals in cell membranes remain in the lipid phase and cause more oxidative damage. It is noteworthy that while vitamin E functions to convert lipid peroxy radicals to lipid peroxide, it is being converted to tocopheroxyl radicals (Tocopherol-O') and requires a regeneration back to tocopherol, in order to prevent unwanted tocopheroxyl-mediated oxidative processes.\(^{(9)}\)

**Vitamin E regeneration system (VERS):** The regeneration of tocopherol is highly integrated (Fig 2) and involves multiple reactions in both aqueous and lipid phases.\(^{(8)}\);\(^{(10)}\)
One of the antioxidation activities of GSH is to indirectly eliminate the reactive tocopherol-O’, which is formed per (EQ 1). At the aqueous surface, GSH donates an electron via H’ to the reactive Tocopherol-O’, and reduce it to the more stable Tocopherol-OH (EQ 3).\(^{(10)}\)

\[
2\text{Tocopherol-O’} + 2\text{GSH} \rightarrow 2\text{Tocopherol-OH} + \text{GSSG} \quad \text{EQ 3}
\]

Vitamin C (ascorbic acid: (AA)) also undergoes a sacrificial 2-electron oxidation to regenerate vitamin E from the tocopheroxyl radical (EQ 4) to Tocopherol-OH.\(^{(11)}\) It occurs in the aqueous phase and involves two single-electron reductions, first yielding the semi-dehydro-ascorbyl radical and ultimately, dehydroascorbic acid (DHA).

\[
2\text{Tocopherol-O’} + \text{AA} \rightarrow \text{DHA} + 2\text{Tocopherol-OH} \quad \text{EQ 4}
\]

The DHA in turn is recycled to ascorbic acid by GSH which in turn is oxidised forming GSSG (EQ 5).\(^{(8)}\)

\[
\text{DHA} + 2\text{GSH} \rightarrow \text{AA} + \text{GSSG} \quad \text{EQ 5}
\]

Ubiquinol (CoQ-H\(_2\)), otherwise known as coenzyme-Q\(_{10}\), has both direct and indirect antioxidant activity in the lipid phase. Indirectly, it acts by regenerating tocopherol from the tocopheroxyl radical as shown in (EQ 6).

\[
2\text{Tocopherol-O’} + \text{CoQ-H}_2 \rightarrow 2\text{Tocopherol-OH} + \text{CoQ} \quad \text{EQ 6}
\]

Ubiquinol directly, breaks the lipid peroxidation chain by donation of electrons and in the process becomes the oxidised ubiquinone (CoQ) shown in reaction (EQ 7).\(^{(8)}\) That is, coenzyme-Q\(_{10}\) is a substitute for vitamin E, though the latter is more abundant.\(^{(12)}\)

\[
2\text{Lipid-O}_2’ + \text{CoQ-H}_2 \rightarrow 2\text{Lipid-O}_2\text{H} + \text{CoQ} \quad \text{EQ 7}
\]

Here, we propose an integrated model of vitamin E regeneration that incorporates the separate reactions outlined above as a ‘vitamin E regeneration system’ (VERS). Figure 2
Fig 2: Illustration of VERS – (Keys: 1-6 = EQ1 – EQ6 respectively; AA = ascorbic acid; DAA = dehydro-ascorbic acid; GR = glutathione reductase; GSH = reduced glutathione; GSSG = oxidised glutathione; LOOH = lipid peroxide; LOO· = lipid peroxyl radical; RC = mitochondrial respiratory chain; TOH = tocopherol (vitamin E); TO· = tocopheroxyl radical)

Figure 2 shows that the situation with antioxidant supplementation is not straightforward. Endogenous GSH and/or micronutrient vitamin C and Coenzyme-Q₁₀ function to prevent vitamin E from exhibiting toxicity. The figure also shows that besides GSH...
regeneration, vitamin E and vitamin C are dependent on GSH for their regeneration. Furthermore, Coenzyme-Q₁₀ is both regenerating as well as supplementing vitamin E.

**The problem: Lack of complete antioxidant profiling – not conflict between negative/neutral observational and positive experimental reports**

To our knowledge, these multiple regenerating reactions have up to now only been studied as separate entities rather than as a complex set of integrated processes. There is no single study that has determined the level of vitamin E together with all of the co-antioxidant (Coenzyme Q₁₀, glutathione and vitamin C) network components that constitute our VERS model. However, there are credible suggestions in the literature that vitamin E supplementation is only necessary for disease conditions. Indeed, when there are adequate levels of coenzyme-Q₁₀, GSH and vitamin C, the respective pathways (number [5], [2] and/or [3] in Figure 2) are capable of regenerating vitamin E for the continuation of its antioxidant activity.

The tendency for vitamin E to show a negative or no effect during disease progression when there are inadequate levels of one or more co-antioxidant can be explained by the importance of the other co-antioxidants in the proposed VERS model as each of them affects the regeneration of Vitamin E as an antioxidant. Thus the controversial findings in the literature suggesting a benefit, no action or a negative effect of Vitamin E supplementation may be related to the state of the total system.

For instance, a critical appraisal of experimental positive results from Yang et al. (2007), observational neutral results from Lonn et al. (2002), and observational negative results from Ward et al. (2007), using figures 1 and 2, showed that these different results are essentially not contradictory. Instead, they are complementary, giving
a more complete picture of the need and limitations of vitamin E as these three studies have administered vitamin E at different stages of OS. Therefore, while the results are justifiable, they do not conflict with one another.

The hypothesis: Algorithm to justify antioxidant vitamin E supplementation in DM

We present an algorithm (Fig. 3), based on published researchers’ opinions or critique of vitamin E studies, as a model for assessing vitamin E activity and efficacy when used as a treatment supplement. The model as displayed in figure 3 points to pertinent questions that can now be addressed to avoid organising a clinical trial that has been identified as biased or limited.

It suggests that for a clinical study that intends to observe the effectiveness of vitamin E, there needs to be a likelihood of OS, which requires laboratory-based evidence. This is imperative, since antioxidant vitamin supplementation is potentially risky if there is no OS.\(^{(15)}\) This is supported by the report of Jialal and Devaraj (2005), who in their review concluded that several confounding factors that account for the lack of benefit of vitamin E are omitted in the majority of trials, including assessment of OS biomarkers.\(^{(20)}\) This step is particularly important because whether, how, and when OS occurs in the progression of cardiovascular complications in diabetes is not straightforward and needs to be demonstrated.\(^{(7)}\) The effectiveness or need for antioxidant vitamin E can be assessed using malondialdehyde and GSH status.\(^{(21)}\)
After determining OS, the next step should be consideration of the disease status. Bearing in mind that in animal studies, diabetes and/or OS is always induced and intervention supplementation is initiated at a pre-determined time, the question here should be whether OS is (1) already advanced, in which case vitamin E therapy is only necessary for management, and would definitely require VERS adequacy; or (2) at onset, in which
case a preventive effect can be possible. The reason for this step is that although vitamin E does quench lipid peroxidation and prevent oxidative damage, there neither any theory nor results that suggest its ability to reverse any cardiovascular damage that may have arisen from previous OS processes. Therefore, since vitamin E supplementation will not reverse vascular damage that may have occurred during its deficiency, considering the disease status is important in order to set a credible goal that is within the capacity of vitamin E antioxidant activity.

The next point of consideration is establishment of vitamin E deficiency and assurance that the problem is not necessarily that of inadequate regenerating co-antioxidants. For this, the vitamin E level has to be determined, as well as its co-antioxidants.\(^{20;22}\)

**Potential implications: Vitamin E supplementation has contraindication**

The importance of considering all co-antioxidants acting within a unified physiological process is that antioxidant interactions are complex and determine whether vitamin E exhibits antioxidant or pro-oxidant activity, which translates to positive or negative effects respectively.\(^{9;13}\) Hence it has been cautioned that no pathway should be arbitrarily assumed to be predominant in disease conditions.\(^8\)

In the situations of any deficient co-antioxidant activity, it is impossible for vitamin E to be effective. Instead, administration of even a normal daily dose of vitamin E will drive (EQ 1) to yield pro-oxidant tocopheroxyl radicals that may not be regenerated early enough. The net effect is increased OS, which is negative and unwanted. In medical practice, this situation simply translates to ‘contraindication for vitamin E therapy’, which can be likened to immunosuppression contraindicating vaccination.\(^{23}\) That is, it can be said that _VERS inadequacy contraindicates vitamin E supplementation as_
immunosuppression contraindicates vaccination. Therefore, pending experimental verification, we speculate that vitamin E is unjustified if VERS components are not adequate.

It is pertinent to differentiate this unjustifiable scenario from risk factor contributions. While the latter refers to factors that have variable predispositions to adverse outcomes, the former refers to factors that surely predispose to adverse outcome and therefore prohibit administration of a particular therapy. For instance, a vitamin E dose greater than 400 IU/day is a risk factor that may or may not exacerbate oxidative stress. This does not necessarily contraindicate administration of vitamin E. However, inadequate levels of regenerating co-antioxidants provide counsel against administration of vitamin E, because even a low dose of the supplement could cause unwanted effects.

Potential tests to assess contraindication - Importance of determining VERS status

Vitamin E is neither a substitute nor a supplement for cellular glutathione. Since cellular glutathione serves as the frontline defence against oxidative damage, the need for vitamin E countering lipid peroxidation has to be evidenced by the usually preceding reduced level of GSH activity and the subsequent increased levels of lipid peroxidation products such as malondialdehyde. Since co-supplementation does not necessarily guarantee effectiveness, the assurance that the proposed co-supplementation of either vitamin C or co-enzyme Q10 will be effective needs to be verified by determination of the levels of co-antioxidants.

It is already known that there are abnormalities in metabolism of ascorbic acid and GSH, as well as defects of chain-breaking antioxidant system in the blood. What this report hypothesizes is articulation of the interactive co-antioxidant network, which we term
VERS, and clarification of the seemingly conflicting reports of previous vitamin E studies. In addition, our novel algorithm indicates that it is important to establish the levels of the co-antioxidant when determining whether vitamin E supplementation will be beneficial or harmful.

**Conclusion**

It is imperative to recognize that there is neither a theoretical justification for vitamin E supplementation when there is inadequate VERS status, nor any theory to support that cardiovascular disease subjects on vitamin E would have their pathology reversed. As a novel application of a known idea, we suggest determining the VERS status to establish and provide the basis for evidence-based prescribing practice for effective vitamin E supplementation. Furthermore, the need for the antioxidant must be justified with evidence of vitamin E deficiency amidst optimal levels of all three regenerating co-antioxidants and a pathological condition that is associated with sustained oxidative stress. An algorithm to guide generation of this evidence is provided.

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