

**Review Article**

# Laboratory evaluations to optimize outcomes of antioxidant nutrition therapy in diabetes management

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Medical nutrition therapy (MNT) guidelines acknowledge the need to identify deficiencies of antioxidant vitamins. However, the guidelines contain that such identification is difficult. Thus, there is evidence that available clinical laboratory tests for antioxidant vitamins C and E are not in perspective in clinical practice. Coenzyme-Q<sub>10</sub> and glutathione tests are also available in research laboratories. These indices are invaluable tools for discrete recommendation and monitoring of antioxidant nutrition therapies. This commentary addresses biomarker insight to what the MNT guidelines consider difficult. The importance and limits of the various dietary antioxidants is overviewed. It puts in perspective how clinical laboratory monitoring of vitamins C and E levels can be used to optimize the outcomes of dietary evaluations for diabetes management. Insight to how to interpret the laboratory results is presented. The importance of this commentary is hinged on the premise that the outcome of dietary therapy can be counter-productive when laboratory evaluation or limitations of the antioxidant nutrients are undermined. (Nwose EU. *North Am J Med Sci* 2009; 1: 137-141).

**Keywords:** Biomarkers, Clinical laboratory monitoring, Medical nutrition therapy guidelines

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## Introduction

There is rationale for dietary therapy in diabetes mellitus (DM). This is shown in the evidence-based practice for Dieticians [1]. Coenzyme-Q<sub>10</sub> (CoQ-H<sub>2</sub>), N-acetylcysteine, vitamin C and vitamin E amongst others have been identified as nutritional ingredients necessary for DM management [2]. This identifies the effects of antioxidants in the management of oxidative damage in DM.

To prevent or slow down the progression of diabetes and its complications, Dieticians evaluate the patient's diet and determine necessary changes. Clinical algorithms have been suggested to guide the use of dietary therapy using laboratory measures [2, 3]. However, the suggestions do not include any identifiable antioxidants. Thus, the crucial factor of oxidative stress (OS) and the basic principle that physiological systems are regulated by metabolic processes is being overlooked.

The inherent and ongoing danger is that the efficacy of medical nutrition therapy is still sub-optimal and the validity still a subject of debate. For instance, while there has been acknowledgement that vitamins C and E can nullify oxidative stress and prevent diabetic cardiovascular complications, there are contrary reports of the risk of hypertension and mortality associated with vitamin E in DM patients [4, 5].

The dangerous debate surrounding antioxidant vitamins was hallmarked by the Guideline of the American Heart Association (AHA) that listed antioxidant vitamin supplements as not recommended for CVD prevention in diabetes, because of risk of harm [6]. Furthermore, the recently published executive summary of revised recommendations from Canada does not contain any statement regarding antioxidant vitamins [7]. This raises the need to clarify how the different antioxidants act in DM, how the widely reported toxicity arises and how the toxicity can be avoided in dietary treatment of diabetes. The objective of this commentary is to offer insights that put in perspective how to optimize outcomes of medical nutrition therapies in diabetes management through laboratory monitoring.

## Review of data and literatures

To achieve the set objective, the following three questions were briefly addressed by brief reviews. (i) How are the choices and outcomes of antioxidant vitamins nutrition determined in research? (ii) How are the choices and outcomes of antioxidant vitamins nutrition determined in clinical practice? (iii) What does the medical nutrition therapy guidelines recommend?

*Review 1: How are the choices and outcomes of antioxidant vitamins nutrition determined in research?* The literatures available on Ovid, PubMed, Science-Direct and Wiley InterScience databases were reviewed similar to the method

of Witte, Clarke and Cleland [8]. This review was with particular interest on the antioxidant vitamins and co-antioxidants that were determined in diabetes study. Therefore vitamins C and E as well as diabetes, CoQ-H<sub>2</sub>, and reduced glutathione (GSH) were used as search terms. The search was limited to English and between June 1996 and June 2006.

The result, which was presented at an international conference [9], shows that none of the opponents or proponents of vitamin E nutrition has evaluated all of the endogenous co-antioxidants that are necessary for optimal efficacy (Table 1). A random review of some of the research protocols showed that every study acknowledged the importance of co-antioxidants interactions, but omitted the laboratory assessment of at least one co-antioxidant component.

**Table 1** Number of published articles containing search terms.

	Ovid	PubMed	S-Direct*	Wiley*
VE	1320	10807	8159	7261
VE + VERS	16	1148	45	23
VE + VERS + DM	3	40	21	23
Determination of VE + VERS	0	0	0	0

Keys: S-Direct\* = Science-Direct; Wiley\* = Wiley InterScience; VE = vitamin E, VERS = vitamin e regeneration system components (comprising vitamin C, CoQ-H<sub>2</sub>, and GSH)

*Review 2: How are the choices and outcomes of antioxidant vitamins nutrition determined in clinical practice?* First, a one year (January to December 2007) archived results were reviewed to ascertain whether the antioxidant tests that were performed in our laboratory are appropriately in perspective. Secondly, the “Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications” was reviewed [10].

As reported in a conference [11], Table 2 presents data of antioxidant vitamins tests that were performed in 2007. A total of 115 vitamin E and 32 vitamin C tests were performed. CoQ-H<sub>2</sub> and GSH levels are not being determined (Table 2). Furthermore, it is interesting to note that these tests were not requested by Nutrition and Dietetic practitioners or for the purpose of nutrition evaluation in DM management.

**Table 2:** Number of antioxidant assessed in 2007 at a reference clinical laboratory

Months	1	2	3	4	5	6	7	8	9	10	11	12
Vitamin C	3	3	1	1	0	3	1	3	1	11	1	4
Vitamin E	1	6	10	9	3	16	6	17	7	27	5	8
CoQ-H <sub>2</sub>	Not tested											
GSH												

In another reported review, laboratory records of all diabetes patients who were followed up for diabetes control in 2008 were audited. It was observed that none of the 10,342 glycaemic index (HbA<sub>1c</sub>) requests received in the year was requested for any antioxidant vitamin [12].

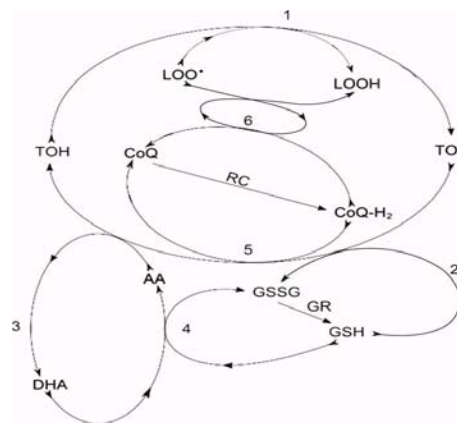
*Review 3: What does the medical nutrition therapy guidelines recommend?* The position statement and recommendations from American Diabetes Association were reviewed [10]. The guidelines indicate amongst others that *if deficiencies of antioxidant vitamins are identified, supplementation can be beneficial, but that this is difficult to ascertain.*

The executive summary of ‘Canadian Diabetes Association 2008 Clinical Practice Guidelines for the Prevention and Management of Diabetes in Canada’ was also reviewed. There is no advice on antioxidant vitamins or its laboratory evaluation [7].

This shows that choices of antioxidant nutrition are not based on any laboratory evaluation of levels of the micronutrients; because the guidelines are silent and/or contain that it is difficult to ascertain. This is evidence of either lack of awareness or a system oversight that requires addressing.

## Discussion

*Antioxidant activities in diabetes pathology:* To correct the possible oversight, it is pertinent to have an overview of antioxidant activities and limitations. This is one of a complex interactions involving feedback and feedforward effect of biochemical processes. The activity of one antioxidant triggers the action of one or more co-antioxidant, but all constitute a kind of closed circuit network (Fig. 1).



**Fig 1** Illustration of vitamin E regeneration system. Keys: 1-6 = EQ1 – EQ6 respectively; AA = ascorbic acid; DAA = dehydro-ascorbic acid; GR = glutathione reductase; GSH = reduced glutathione; GSSG = oxidized glutathione; LOOH = lipid peroxide; LOO• = lipid peroxy radical; RC = mitochondrial respiratory chain; TOH = tocopherol (vitamin E); TO• = tocopheroxyl radical [13].

**Importance and limit of reduced glutathione (GSH):** In DM, the frontline cellular antioxidant is GSH, which is involved in recycling both vitamins C and E [14, 15]. When there is inadequate level of GSH, both vitamins E and C are inadequately regenerated. A pathophysiological effect is that un-regenerated radicals of the vitamins will exercise their pro-oxidant activity and exacerbate OS (Fig. 1). The manifestation is negative observation that invalidates an otherwise valid nutrition therapy.

Therefore, when vitamin E supplementation is prescribed without assessment of GSH level, two sources of error are inherent. Firstly, evidence is not established whether the person's actual need is vitamin E. It could be that the frontline antioxidant is what is lacking. Secondly, the adequacy of the antioxidant that will regenerate vitamins C and E from their pro-oxidant states is being undermined.

This is the basis of support for N-acetylcysteine in diabetes management, to supplement of GSH [2]. The limit of GSH level is occasioned by the glutathione enzymes' activities [16, 17].

**Vitamin E activity:** The antioxidation reaction of Vitamin E (Tocopherol-OH) involves loss of electron *via* donation of a hydrogen atom (H<sup>•</sup>). It then becomes a pro-oxidant molecule (Tocopherol-O<sup>•</sup>) that is capable of initiating OS by a process of tocopherol-mediated peroxidation [18].

Tocopherol-mediated peroxidation is prevented by complex feedback activities of CoQ-H<sub>2</sub>, GSH and vitamin C [13, 19]. When there is deficiency in one or more of these co-antioxidants (Fig. 1), the feedforward OS that occurs explains the reported toxicity of vitamin E [15].

**Importance and limit of vitamin C:** Vitamin C (ascorbic acid) fundamentally acts in the regeneration of vitamin E [19]. Therefore, its therapeutic use as an antioxidant is only necessary if there is either evidence of inadequate recycling of vitamin E, or foreseeable need to backup for intended vitamin E supplementation.

Ascorbic acid acts first by donating one electron and thereafter becomes dehydroascorbic acid, which has a pro-oxidant property [20]. It requires GSH for its normal recycling (Fig. 1). Otherwise, the pro-oxidant activity of dehydroascorbic acid causes OS, which explains the reports of toxicity and casts doubt on the validity of vitamin C therapy.

**Importance and limit of CoQ-H<sub>2</sub>:** A reduced rate of regeneration of vitamin E by GSH and/or vitamin C is accompanied by a deficiency of vitamin E to scavenge the lipid peroxide. As a feedback mechanism, the CoQ-H<sub>2</sub> activity will tend to increase [21]. This triggers a feedback whereby CoQ-H<sub>2</sub> tends to increase its rate of scavenging for the accumulating lipid peroxides (Fig. 1).

Although CoQ-H<sub>2</sub> may be indispensable as a mitochondrial antioxidant and is useful for the regeneration of vitamin E (Fig. 1), its rate of reaction shifts during vitamin E deficiency and cause its sub-optimal activities in the mitochondria [22]. Meanwhile, there is the paradox of increased ROS production in the respiratory chain, which contributes to oxidative mitochondrial damage, thereby exacerbating DM complications [23].

In summary, antioxidant interactions constitute a complex factor that determines whether a therapeutic dietary recommendation yields a positive or negative outcome [18, 19]. It is a vital precaution that no single component is arbitrarily assumed to be predominant in disease conditions [17]. This draws attention to the notion that giving vitamins C and E supplements at the onset of diabetes is preventive.

The fallacy or omission in this belief is that the limitation of vitamin C in regenerating vitamin E is overlooked. If any of the other co-antioxidants is deficient, it is impossible for vitamin C and/or E to be very effective. Instead, administration of even a normal daily dose of the antioxidant vitamin will yield pro-oxidants that may not be regenerated well enough. The net effect is increased OS, which is negative and unwanted [24]. Thereby casting doubt about the validity of the antioxidant supplement or dietary regimen.

*Insights to optimize medical nutritional therapies with laboratory assessment:* Firstly, the impression that identification of deficiencies of antioxidant vitamin level is difficult to ascertain needs to be corrected. Validated methods for *in vitro* diagnostic use exist for vitamins C and E (Table 1). In Australia, the tests can be done at no cost to the patient, because they are listed for bulk billing. The other strongly related co-antioxidants, CoQ-H<sub>2</sub> and GSH, are available in some research laboratories, but still require standardization for *in vitro* diagnostic use. For the mean time, the available vitamins C and E tests can be used to ascertain the levels of antioxidant vitamins, which the current guideline acknowledges to be important but did not recommend.

Secondly, it is pertinent to note the following points on how to interpret and optimize the laboratory tests.

- If laboratory result indicates normal level of vitamin C and/or E, it provides evidence base NOT to recommend the antioxidant vitamin nutrition/supplement.
- If laboratory result flags low level of vitamin C and/or E, one of two scenarios is probable.
  - a. The antioxidant vitamin may be in abundance, but existing in its pro-oxidant form and requires to be recycled. That is, it may be that the regenerating co-antioxidant is deficient. Imagine that a person who was tested for vitamin C showed a low level result. If there is deficient GSH, any recommended nutritional or supplementary vitamin C

- would be converted to additional DHA without being recycled. Thus, follow up laboratory tests result will flag low level.
- b. The antioxidant may be truly deficient and requires supplementation. If there is sufficient GSH, the recommended nutritional or supplementary vitamin C would be converted to DHA, but adequately recycled in a feedback response. Thus, follow up laboratory tests result will indicate improved or normal level.
  - Assess the levels of both vitamins C and E. The data presented shows that in the month of May 2007, three persons were tested for vitamin E; none of whom was tested for vitamin C (Table 2). If the test were requested for medical nutrition purposes, it would benefit to determine whether a deficiency of vitamin E is concomitant with deficiency of vitamin C. Such determination will form evidence base to take one of three decisions
    - a. Start with an initial supplementation of vitamin C and evaluate how much of vitamin E has been recycled (from its pro-oxidant state). During follow up, non-improvement in vitamin E levels with improvement in vitamin C should be affirmation of deficiency of the former. However, improvement in vitamin E levels with no improvement in vitamin C should suggest deficient recycling of the latter, which in turn suggest a requirement for N-acetylcysteine nutrient to boost GSH synthesis.
    - b. Vitamins C and E nutrients and/or supplements could be given with follow up tests to evaluate improvement in levels. A repeat observation of low level results would be clear indication of failed regeneration system (Fig. 1). That is, it will be indirect indication of deficient levels of other co-antioxidants, which will then form evidence base for the need of nutritional therapies other than vitamins C or E.
    - c. Concomitant deficiencies of vitamins C and E being probable indication of failed regeneration system can be attended to. In this case, it has to be noted that the vitamins may be much in the pro-oxidant forms and requiring regeneration. Therefore, a discrete combination of food that contains CoQ-H<sub>2</sub> and N-acetylcysteine may be what is required to improve recycling. If the probability is true, the follow up evaluation tests should present improved levels of vitamins C and E.

These are suggestions of how and when clinical laboratory monitoring can optimize the outcomes of dietary evaluations. The summary insight is that in an occasion where the laboratory results of vitamin C and/or E flags low, any recommended nutritional therapy should necessarily be monitored with follow-up laboratory evaluations.

## Conclusions

Dietitians have played and are still playing crucial roles in diabetes and diabetic complications programs [25, 26]. Clinical algorithms have been proposed to guide the use of medical nutrition therapy using validated laboratory measures of glycaemic control, insulin sensitivity, and beta-cell reserve [2, 27]. However, these algorithms and laboratory measures provide limited advice [28]. Hence, patients are often given incorrect and inappropriate dietary advice by their health consultants [27]. What this article contributes is that the process of dietary evaluation for patients will benefit from laboratory assessment of the patient's need for specific nutritional antioxidants. While the effect of dietary therapy in diabetes management is not in doubt, the outcome of dietary evaluation and therapy can be in doubt when the limitations of the antioxidant nutrients are undermined. Performing laboratory test of antioxidant status of patients during dietician's assessment would be invaluable protocol to optimize outcomes.

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