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Review Article

Micronutrient-Induced Activation of Nrf2 and -Elevation of Intracellular Antioxidants for Reducing Oxidative Stress and Chronic Inflammation in Diabetes

Abstract

Despite extensive research and dietary and lifestyle recommendations, the incidence of diabetes continues to increase. Diabetic medications have been useful in controlling blood levels of glucose; however, in general, the disease continues to progress rather slowly, and eventually leads to diabetic-related complications. Analysis of published studies suggests that increased oxidative stress and chronic inflammation initiate and promote the progression of diabetes and diabetic-related complications. Classical anti-diabetic measures are aimed at control of glucose levels and do not reduce these factors. Therefore, together with standard care and adoption of appropriate diet and lifestyle, attenuation of these biochemical defects may have utility in the prevention, and in combination with standard care, for the improved management of diabetes. The question arises how to maximally and at the same time reduce oxidative stress and inflammation. This review describes how simultaneous attenuation of excess oxidative stress and inflammation may be enabled. This briefly discusses diverse actions and cellular distributions of different antioxidants in the body, and describes the effects of individual antioxidants in prevention and management of diabetes. It proposes that increasing the levels of antioxidant enzymes and dietary and endogenous antioxidant compounds at the same time is needed to decrease oxidative damage and chronic inflammation. An oral supplementation can increase the levels of antioxidant compounds; however, increasing the levels of antioxidant enzymes requires activation of Nrf2. This review discusses activation of Nrf2, and proposes a mixture of micronutrients that enhances the levels of antioxidant enzymes by activating the Nrf2 pathway, and also increases intracellular levels of antioxidant compounds. This combination is likely to have utility in patients with diabetes.

Introduction

Diabetes mellitus is characterized by hyperglycemia that results from insufficient or lack of production of insulin by the pancreas. Insulin resistance develops due to oxidative damage to the glucose transporter proteins or insulin receptors can lead to hyperglycemia. Diabetes has reached epidemic proportions throughout the world. In the USA, about 19 million people had diabetes in 2010; this number increased to 21 million in 2014. The number of undiagnosed case also rose from 7 million in 2010 to 8.1 million in 2014 (Center for Disease Prevention, 2014 National Diabetes Statistic report). The cost of diabetes and pre-diabetes care in the USA is \$322.00 billion per year (American Diabetic Association, 2016). Despite extensive basic and clinical studies, the number of diabetes cases continues to increase. One of the reasons could be that the current recommendations, such as, losing weight, doing daily moderate exercise, eating

a balanced diet, and stopping tobacco smoking are not being followed by most people.

The role of reactive oxygen species (ROS) in normal cell functions is complex depending upon their levels in the cells. Low levels of ROS activate cell-signaling pathways that are essential for initiating biochemical and physiological processes necessary for the survival and functions of the cells. A review has discussed these issues in detail [1]. On the other hand, high levels of ROS referred as oxidative stress induces cellular damage including DNA, RNA protein and membranes. Analysis of published studies has concluded that increased oxidative stress and chronic inflammation are primarily responsible for the initiation and progression of diabetes as well as for the development of diabetes-related complications [2]. Since then, several studies confirming the role of oxidative stress in diabetes have also been published [3-10]. Increased oxidative

damage, if not repaired, can lead to chronic inflammation that releases free radicals, pro-inflammatory cytokines, complement proteins, adhesion molecules and prostaglandins, all of which are toxic to cells. This issue has been discussed in a previous review [2]. Since then, additional studies on the role of chronic inflammation in diabetes and diabetic-related complications have been published [11–18]. Strongest support for the role of oxidative stress comes from the report that markers of oxidative damage were elevated in pre-diabetic patients [6], and in the parents of diabetic as well as children with type 1 diabetes [19,20].

Increased oxidative stress and chronic inflammation can damage the pancreas, glucose transporter proteins and insulin receptors that contribute to hyperglycemia. If the pancreas is producing sufficient amounts of insulin, oxidative damage to the glucose transporter proteins or insulin receptors may cause tissue resistance to insulin leading to hyperglycemia. Since these biochemical defects initiate and promote diabetes, reducing these biochemical defects may be one of the rational choices for the prevention, and in combination with standard treatment, for the improved management of diabetes.

Despite advances in medications for controlling blood glucose levels, the disease slowly continues to progress. This may in part be because these medications do not adequately address the issues of increased oxidative stress and chronic inflammation.

This review briefly discusses diverse actions and cellular distributions of different antioxidants in the body, and describes the effects of individual antioxidants in prevention and management of diabetes. It proposes that increasing the levels of antioxidant enzymes and dietary and endogenous antioxidant compounds at the same time is needed to decrease oxidative stress and chronic inflammation. An oral supplementation can increase the levels of antioxidant compounds; however, increasing the levels of antioxidant enzymes requires activation of Nrf2. This review discusses how an appropriate mixture of micronutrients can simultaneously enable activation of Nrf2 pathway, leading to elevation of antioxidant enzymes, and directly enhance levels of intracellular antioxidants.

Diverse actions and cellular distributions of antioxidants in the body

The body protects against oxidative damage by antioxidant compounds derived from the diet as well as made in the body, and antioxidant enzymes made in the body. Antioxidant compounds reduce oxidative stress by a mechanism in part different from that of antioxidant enzymes. They destroy free radicals by donating electron to unpaired state of the free radicals. On the other hand, antioxidant enzymes destroy free radicals by catalysis (converting them to the harmless molecules such as to water and oxygen). Some antioxidant compounds also reduce chronic inflammation [21–25]. Different antioxidants have different affinities for free radicals depending upon their location in the cellular compartments. Water-soluble antioxidants such as vitamin C and glutathione protect molecules in the aqueous environment of the cells, whereas lipid soluble antioxidants such as vitamin A and

vitamin E protect molecules in the lipid environment of the cells. Vitamin E was more effective in quenching free radicals in a reduced oxygenated cellular environment, whereas vitamin C and vitamin A were more effective in a higher oxygenated environment of the cells [26]. Vitamin C is important for recycling the oxidized form of vitamin E to the antioxidant form [27]. In addition, antioxidants produce cell protective proteins by upregulating or downregulating different microRNAs [28]. For example, some antioxidant can activate Nrf2 by upregulating miR-200a that inhibits its target protein Keap1 [29], whereas others activate Nrf2 by downregulating miR-21 that binds with 3'-UTR Nrf2 mRNA [29].

Effects a single antioxidant compound on type 2 diabetes

Because of the complexity and different mechanisms of action and cellular distributions of different antioxidants, it is not possible to simultaneously reduce oxidative stress and inflammation by a single antioxidant or anti-inflammatory agent. Nevertheless, previous studies have investigated the effects of using a single antioxidant primarily in animal models of diabetes. These antioxidants include vitamin A, vitamin C, vitamin D, vitamin E, alpha-lipoic acid, n-acetylcysteine, L-carnitine, coenzyme Q10, folic acid, thymine, omega-3-fatty acids, and chromium. The studies on the effects of these individual antioxidants in diabetes have been previously reviewed [2]. Treatment with a single antioxidant was found to lead to some beneficial effects in animal models and in patients with diabetes. Since then, additional studies on the effects of a single antioxidant, such as sulphoraphane, riboflavin, curcumin, resveratrol, alpha-lipoic acid, vitamin C, omega-3-fatty acids, and coenzyme Q10 on animal models of diabetes and human diabetes have produced similar results [30–37]. In some studies supplementation with resveratrol had no effect on glycemic control [38], selenium had no effect on insulin resistance [39], and chromium compound had no effect on glycated hemoglobin (A1C) in patients with type 2 diabetes [40]. One of the reasons for the inconsistent benefits with a single antioxidant in patients with diabetes could be that a single antioxidant failed to simultaneously reduce oxidative stress inflammation in these patients. Other reasons could be that a single antioxidant in high oxidative environment of diabetic patients is oxidized and then acts as a pro-oxidant rather than as an antioxidant.

Based on the differential actions and distributions of antioxidant enzymes and antioxidant compounds, simultaneous increase in the levels of antioxidant enzymes through the activation of Nrf2/ARE (nuclear transcriptional factor-2/antioxidant response element) pathway, together with administration of dietary and endogenous antioxidant compounds may be necessary for optimally reducing oxidative stress and chronic inflammation in Alzheimer's disease [41]. A similar strategy may be needed for reducing these biochemical defects in patients with diabetes. The levels of antioxidant compounds can easily be enhanced by an oral supplementation; however, increasing the levels of antioxidant enzymes is complex requiring an activation of the Nrf2/ARE pathway.

Nuclear transcriptional factor-2 (Nrf2)

The Nrf2 (nuclear factor-erythroid-2-related factor 2) belongs to the Cap¹N¹Collar (CNC) family that contains a conserved basic leucine zipper (bZIP) transcriptional factor [42]. Under physiological condition, Nrf2 is associated with Kelch-like ECH associated protein 1 (Keap1) that acts as an inhibitor of Nrf2 [43]. Keap1 protein serves as an adaptor to link Nrf2 to the ubiquitin ligase Cui-Rbx1 complex for degradation by proteasomes and maintains the steady levels of Nrf2 in the cytoplasm. Nrf2-keap1 complex is primarily located in the cytoplasm. Keap1 acts as a sensor for ROS/electrophilic stress.

Activation of Nrf2

Activation of Nrf2 during acute oxidative stress: Acute oxidative stress occurs during aerobic exercise, treatment of cells with chemicals such as H₂O₂ for a short period of time [44], and treatment of animals with the donors of free radicals, such as 3,3',5'-triiodo-L-thyronine, a thyroid hormone for a few hours [45]. During acute oxidative stress, ROS (reactive oxygen species) activates Nrf2 which then dissociates itself from Keap1-Cui-Rbx1 complex and translocates in the nucleus where it heterodimerizes with a small Maf protein and binds with ARE (antioxidant response element) leading to increased expression of target genes coding for several cytoprotective enzymes including antioxidant enzymes and phase-2-detoxifying enzymes [46-48]. Activation of Nrf2 by a ROS-dependent mechanism is effective in reducing oxidative damage only under acute oxidative stress.

Activation of Nrf2 during chronic oxidative stress: In contrast to acute oxidative stress, Nrf2 becomes resistant to ROS during chronic oxidative stress in neurodegenerative disease [49-51], suggesting that activation of Nrf2 by a ROS-independent mechanism exists. This is also confirmed in patients with diabetes who show increased oxidative stress causing cellular damage continues to occur despite the presence of Nrf2 [2]. The issue then is how to activate ROS-resistant Nrf2 in patients with diabetes.

Antioxidants regulating activation of Nrf2 during acute oxidative stress

ROS generated during acute oxidative stress are essential for activating Nrf2. Pre-treatment with antioxidants such as NAC (n-acetylcysteine) during acute oxidative stress prevented activation of Nrf2 [44,45]. It appears that pretreatment with an antioxidant prevented oxidative damage by scavenging all ROS. Since ROS was not available, Nrf2 was not activated. These results should not be interpreted to mean that lack of activation of Nrf2 in the presence of an antioxidant is harmful. In the studies described above, NAC directly substituted for Nrf2 by removing free radicals during acute oxidative stress.

Antioxidants regulating activation of Nrf2 during chronic oxidative stress

Antioxidant compounds activate Nrf2 during chronic stress without the need for ROS.

Some examples of such antioxidant compounds include vitamin E and genistein [52], alpha-lipoic acid [53], curcumin [54], resveratrol [55,56], omega-3-fatty acids [57,58], glutathione [59], NAC [60], and coenzyme Q10 [61]. Several plant-derived phytochemicals, such as epigallocatechin-3-gallate, carestol, kahweol, cinnamonyl-based compounds, zerumbone, lycopene and carnosol [42,62,63], genistein [52], allicin, a major organosulfur compound found in garlic [64], sulforaphane, a organosulfur compound, found in cruciferous vegetables [65], and kavalactones (methysticin, kavain and yangonin) [66]. The exact reasons for the activation of Nrf2 by antioxidant compounds in the absence of ROS are unclear. Analysis of previous studies suggests that antioxidants may activate Nrf2 by altering the expression levels of microRNAs [28].

Regulation of activation of Nrf2 by MicroRNAs

MicroRNAs are evolutionary conserved small non-coding endogenous single-stranded RNAs of approximately 22 nucleotides in length, and are present in all living organisms including humans [67-70]. Each miRNA binds with the 3'-UTR (3'-untranslated region) of a specific mRNA causing its degradation, thereby, reducing the formation of its target protein [69]. The role of microRNAs in regulating the activation of Nrf2 in diabetes is briefly described here.

Diabetic mice with nephropathy had reduced expression levels of miR-200a and increased expression of miR-21 [29]. MiR-21 binds with the 3'-UTR mRNA of Nrf2; leading to decreased expression of Nrf2. Thus reduced expression of miR-21 would increase the levels of Nrf2. Treatment of mice with curcumin analog C66 that exhibits antioxidant and anti-inflammation activities increased the expression of renal miR-200a that inhibited Keap1, an inhibitor of Nrf2, which allows activation of Nrf2. Treatment of mice with the curcumin analog C66 reduced the expression of miR21 that increases the activation of Nrf2. These results show that increased expression levels of miR-200a enhances Nrf2 activation by inhibiting Keap1 levels, whereas decreased expression levels of miR-21 increases the levels of Nrf2 by reducing its binding to the Nrf2 mRNA [29].

Binding of Nrf2 with ARE in the nucleus

Activation of Nrf2 by ROS-dependent or-independent mechanisms alone is not sufficient to increase the levels of antioxidant enzymes and phase-2-detoxifying enzymes. Activated Nrf2 must bind with ARE in the nucleus in order to increase the expression of many target genes coding cytoprotective enzymes including antioxidant enzymes. This binding ability of activated Nrf2 with ARE in the nucleus was impaired in aged rats [53]. It is unknown whether the binding ability of activated Nrf2 with ARE in the nucleus is impaired in patients with diabetes.

The above studies suggested that activation of Nrf2 would be a useful target for developing agents or drugs for the prevention and improved management of diabetes and diabetic-related complications [71,72]. The levels of antioxidant enzymes and

intracellular levels of antioxidant compounds decreased in both animal models of diabetes and in patients with diabetes [73-76]. In order to restore to more normal balance of intracellular defenses, both of these levels should be elevated together. Thus, activation of Nrf2 by a single antioxidant commonly used in the clinical studies may not be sufficient to optimally reduce oxidative stress and chronic inflammation.

Proposed micronutrient mixture for optimally reducing oxidative stress and chronic inflammation

Based on the differential mechanisms of action and cellular distributions of various antioxidants, a micronutrient mixture containing multiple ingredients is proposed. The contents of this mixture include vitamin A, natural mixed carotenoids, vitamin C, vitamin E, curcumin, resveratrol, alpha-lipoic acid, L-carnitine, coenzyme Q10, a synthetic antioxidant N-acetylcysteine (NAC), vitamin D, all B-vitamins, and omega-3-fatty acids. This mixture may reduce oxidative stress by simultaneously enhancing the levels of antioxidant enzymes through activation of the Nrf2 pathway as well as intracellular levels of antioxidant compounds.

Activation of Nrf2 also suppresses chronic inflammation [77,78]. Some individual antioxidant compounds also reduce chronic inflammation [21-25,79]. Therefore, the proposed micronutrient mixture may also serve to decrease chronic inflammation. The postulated pathways by which diabetes pathology can be restrained, are shown in Figure 1.

Prevention of diabetes

Primary prevention of type 2 diabetes: The purpose of primary prevention is to protect healthy people from

developing diabetes type 2. Individuals who are obese with no pre-diabetic conditions are suitable for the primary prevention. The proposed mixture of micronutrients may be effective in reducing the incidence of type 2 diabetes by reducing chronic oxidative stress and inflammation.

Primary Prevention of type 1 diabetes: At present, there are no clear therapeutic approaches to delay the onset of symptoms in children with a family history of diabetes. The mixture of micronutrients described may be effective in delaying the onset of symptoms of diabetes in such children. This possibility is indirectly supported by a laboratory experiment described here.

The gene HOP (TUM-1) is essential for the development of *Drosophila melanogaster* (fruit fly). Insertion of a mutated HOP (TUM-1) markedly increases the risk of developing a leukemia-like tumor in female flies. In collaboration with Dr. Bhattacharya of NASA, Moffat Field, CA, we observed that whole-body proton irradiation of these flies with proton radiation dramatically increased the incidence of cancer compared to that observed in un-irradiated flies. Treatment with a mixture of antioxidants before and after irradiation blocked the formation of proton radiation-induced cancer in these flies [2]. This finding is of particular interest, because it suggests that heritable genetic basis of the disease can be prevented by antioxidant treatment. The results of this study cannot be extrapolated to the genetic disease in humans.

Secondary prevention of diabetes: The purpose of secondary prevention is to stop or slow the progression of diabetes. Individuals who are pre-diabetic or those who have a family history of diabetes but have not developed symptoms of the disease can be included in secondary prevention. The proposed mixture of micronutrients may be effective in reducing the incidence of diabetes by simultaneously preventing oxidative stress and chronic inflammation.

Improved management of diabetes: Patients with established diabetes are generally on one or more standard medications for improving the treatment of this disease. Combined use of the described proposed described micronutrient mixture in combination with standard therapy may be more effective than confining treatment to maintaining appropriate blood glucose levels.

Diet and lifestyle recommendations: Diet containing plenty of fruits, non-starchy vegetables, complex carbohydrate, and protein, and avoiding sugary drinks are recommended. Lifestyle recommendations include, maintaining normal weight for one's age and height, refraining from smoking, minimizing caffeine consumption, reducing stress by meditation, yoga, vacation, and daily moderate exercise for 30 minutes. The website of American Diabetes Association is recommended for detailed guidelines concerning diet and lifestyle.

Conclusion

Several studies suggest that increased oxidative stress and chronic inflammation play a key role in the initiation and progression of diabetes. Therefore attenuation of these biochemical defects simultaneously might be needed to prevent and improve the management of diabetes. The body's

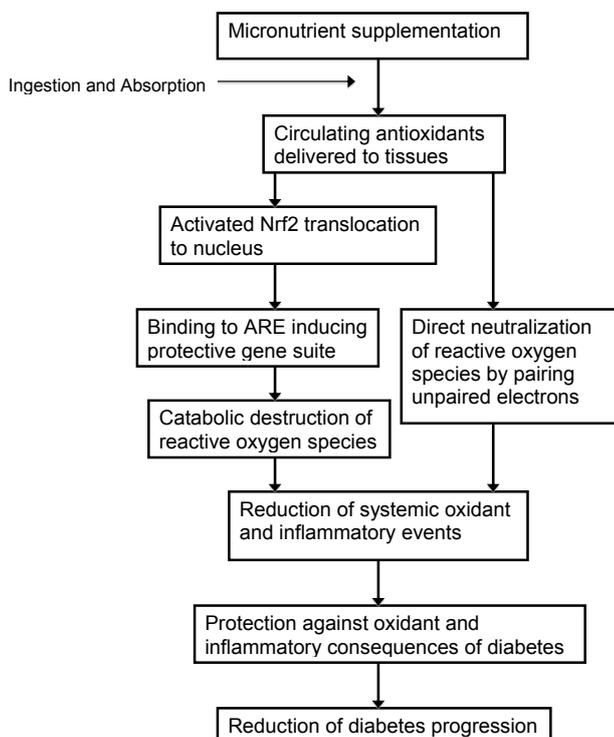


Figure 1: Postulated trajectory of beneficial effects of a micronutrient blend on common aspects of diabetes pathology.

defense system against oxidative stress consists of antioxidant enzymes and dietary and endogenous antioxidant compounds. During the extended chronic oxidative stress found patients with diabetes, intracellular antioxidant compounds as well as antioxidant enzymes are present at reduced levels. Single antioxidant compound or antioxidant enzyme mimetic has been commonly used in animal and human studies and these may not achieve optimal benefit in prevention or diabetes treatment. The proposed micronutrient mixture may be more effective means of reducing oxidative stress and chronic inflammation. The multifocal approach of simultaneous enhancement of levels of antioxidant enzymes through activating the Nrf2/ARE pathway, together with increased presence of antioxidant compounds, ensures the broadest protective spectrum for diabetic control.

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Conflicts

The author is a Chief Scientific Officer of Engage Global, Utah.

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