**INVITED REVIEW**

# Oxidative stress and the role of antioxidative treatment in diabetes mellitus

**Dragana Nikolic[1], Julijana Stanimirovic[2], Predrag Bjelogrlic[3], Esma R. Isenovic[2]**

*[1]Biomedical Department of Internal Medicine and Medical Specialties, University of Palermo,* ***Italy***
*[2]Institute Vinca, Laboratory of Radiobiology and Molecular Genetics, University of Belgrade, Belgrade,* ***Serbia***
*[3]School of Medicine, Clinical Skills, University of St. Andrews, Scotland,* ***United Kingdom***

**Received** March 3, 2014
**Accepted** March 7, 2014

**Published Online** March 28, 2014

**DOI** 10.5455/oams.070314.rv.014

**Corresponding Author**
Esma R. Isenovic
Laboratory of Radiobiology and Molecular Genetics,
University of Belgrade,
P.O. Box 522, Belgrade, Serbia.
isenovic@yahoo.com

**Key Words**
Antioxidative agents; Diabetes Mellitus; Free radicals; Oxidative stress

**Abstract**

It is well known that increased free radical (FR) production or decreased activity of antioxidative system (AOS) lead to an imbalance between pro-oxidants and antioxidants called oxidative stress (OxS). Oxidative stress is involved in numerous diseases including diabetes mellitus (DM).

Elevated blood glucose level and other biochemical disorders accompanied with an inappropriate insulin secretion or improper insulin action are known features of DM. The antioxidative enzyme catalase (CAT) diminishes the production of hydrogen peroxide which is highly toxic for pancreatic cells. The increased activity of this enzyme found in DM type 1 (DMT1) patients signifies the importance of OxS in the pathogenesis of this autoimmune disease with excessive OxS. Additionally, hyperglycemia induces the generation of highly reactive FR and leads to the development of OxS which accelerates the development of DM and its complications associated to the decreased activity of AOS. It is important to point out that high doses of antioxidant agents could paradoxically have pro-oxidant effect.

In this article, we present literature data related to relationship between OxS and DM with focus on non-enzymatic antioxidants as a potential novel therapeutical approach in treatment of DM. Dietary supplementation with antioxidant nutritional factors such as micronutrients and vitamins could be used as a novel strategy in both prevention and control of DM type 2 (DMT2).



## INTRODUCTION

In physiological conditions, oxidative stress (OxS) occurs in all cells which breathe and on this way release free radicals (FR), which can be harmful for the organism, while the FR occurred in pathological conditions are responsible for the damage of biomolecules [1]. During evolution, in response to the creation of FR, protective antioxidant defense mechanisms have been created. In a healthy body, there is a balance between oxidative processes and its antioxidant capacity; but when the equilibrium is disturbed, biologically 'hyper'-active molecules of generated OxS act as signaling agents through various pathways called "redox signaling" [2].

It is believed that the OxS is one of the important factors responsible for cell disorders in diabetes mellitus (DM), firstly initiated by hyperglycemia [3-5]; however, some authors indicate no direct link between hyperglycemia and OxS [6]. The pathogenesis of diabetic complications is related to the increased production in FR [7] and decreased antioxidants [8]. In this context, the mechanisms that may contribute to the formation of reactive oxygen species (ROS) in DM type 2 (DMT2) include also increased non-enzymatic glycosylation [9], glucose auto-oxidation, metabolic stress, levels of mediators of inflammation and antioxidant defense status, that overall lead to dysfunction and cell damage [10]. The main part of the contents of ROS and reactive nitrogen species (RNS), are superoxide ($O_2$•$^-$), hydroxyl (•OH), peroxy (ROO•$^-$), and nitric oxide free radical (•NO) and products of the reactions of these FR, such as hydrogen peroxide ($H_2O_2$) and and peroxynitrite ($ONOO^-$) [11]. As known, $O_2$•$^-$ may cause an increased production of hydroperoxyl radicals and $H_2O_2$ in the presence of superoxide dismutase (SOD), which easily cross cell membranes and initiate oxidative reactions [10]. Very similar happened during lipid peroxidation where lipids are oxidized by FR produced in diabetes. Further, catalase (CAT) and glutathione peroxidase (GSH-Px) are able to convert (reduce) $H_2O_2$ to water ($H_2O$) and oxygen ($O_2$). In the presence of high ferric iron ($Fe^{3+}$), $H_2O_2$ can be reduced to the generation of •OH, a mechanism known as the "Fenton reaction", but also by Haber-Weiss reaction [12], leading to the conversion of $O_2$•$^-$ to •OH.

Some of the drugs currently used in treating DM have been reported to have antioxidant properties [13-15]. However, it is not clear whether such effects are mediated by control of glucose or by the drug itself

[16, 17]. On the other hand, therapeutic methods that directly target the reduction in toxicity of OxS in vascular cells could be a therapeutic approach in patients with DM, in addition to treatments that regulate glucose levels [18]. In this context, treatment with substances which act as antioxidants, in the high-glucose conditions, showed that they inhibit a production of FR and reduce OxS as well as cell damages. Therefore, antioxidants are themselves potential therapeutics in DM treatment [18, 19].

This review was undertaken in order to summarize current knowledge about OxS in DM including possible signaling pathways, with focus on non-enzymatic antioxidants as potential novel approach in the treatment of this disease.

## OXIDATIVE STRESS AND DIABETES

There is increasing evidence that OxS plays a major role in the onset and progression of diabetes, and even its complications [20, 21]. Due to the lack of regulation, a high amount of glucose in the blood increases oxygen and releases $O_2\bullet^-$ which easily reacts with the present nitric oxide (•NO) disabling its action as endothelial vasodilator [19, 22]. Consequently, there is a reduction in endothelium-dependent relaxation and cell synthesis in the wall of blood vessels, resulting in micro- and macro-pathological changes [23, 24]. Hyperglycemia, increases the levels of free fatty acids (FFA), and together with hyperinsulinemia lead to increased production of ROS and RNS [25]. ROS and RNS activate nuclear factor-kappaB (NF-κB), a pro-inflammatory transcription factor, that further cause a signaling cascade leading to a continued synthesis of oxidative species and to inflammation [26]. Increased FFA causes dysfunction by two mechanisms: ***(1)*** the activation of peroxisome proliferator-activated receptor (PPAR)-α which hampers mitochondrial oxidative phosphorylation; and ***(2)*** the production of lipotoxic substances during FFA metabolism leading to opening of $K^+$ channels which impairs $Ca^{2+}$ homeostasis.

Diminished expression of the pyruvate dehydrogenase results in the accumulation of glycolytic intermediate and ceramide inducing apoptosis and consequently leads to cardiomyopathy in DM [21]. Further, hyperglycemia modifies the redox balance through the polyol pathway (reducing glucose to sorbitol, with subsequent decreases in NADPH and reduced glutathione), activates oxidases, and interferes with the mitochondrial electron transport chain [27]. These processes may trigger various signaling cascades, such as activation of protein kinase C, hexosamine pathway that further increase the synthesis of ROS [10, 28]. Non-enzymatically, glucose auto-oxidation generates hydroxyl radicals and leads to the formation of glycation end products (AGEs pathway) that influence the transcription of pro-inflammatory genes to promote OxS [10, 27, 29]. Also, excessive production of FR in hyperglycemia state is ascribed to auto-oxidation of glucose, non-enzymatic glycation of proteins, activation of NAD(P)H oxidases and nitric oxide synthase [30, 31]. Different signaling mechanisms have been described in different complications of DM such as cardiac pathophysiology, renal injury, liver dysfunction [21].

It has been reported that plasma levels of extracellular (EC)-SOD is associated with insulin resistance in DM and its concentrations are significantly higher in diabetic subjects [32]. Further, serum EC-SOD levels positively correlate with the severity of diabetic vascular complications, such as nephropathy and retinopathy [33]. Recent *in vivo* study reported that diabetic skin tissues express a relatively small amount of EC-SOD protein that may be related to elevated ROS production [32].

## DIABETES MELLITUS AND ANTIOXIDANTS

It is considered that a normalization of activity of any OxS markers, such as enzymes, thiobarbituric acid reactive substances (TBARS) and FR, and finally the balance of FR/removal, represent an effective way to reduce the harmful effects of ROS [34, 35]. Based on the latest results, it is clear that the goal is to block the formation of ROS by antioxidants and those results suggest the need for possible use of antioxidants in the treatment of DM. Even more it has been suggested that antioxidant therapy may inhibit the onset of DM and also prevent the development of DM complications [36, 37].

The HOPE (Heart Outcomes Prevention Evaluation trial) study is the largest study dealt with the use of antioxidants in DM. This study has lasted for 4.5 years, and demonstrated that ramipril (a drug used for the treatment of hypertension and heart failure) decreases the possibility of occurrence of diabetic nephropathy in DM patients, as opposed to vitamin E, which did not lead to a significant reduction of cardiovascular risk [19, 38]. Regarding vitamin E several epidemiological studies demonstrated its inverse association with markers of oxidation, inflammation, and DMT2 incidence [39], although other studies did not support such findings [40, 41], including the Women's Health Study [42]. The European Prospective Investigation of Cancer-Norfolk Prospective Study investigated association between fruit and vegetable intake and plasma levels of vitamin C with risk of DMT2, during a 12-year follow-up of 735 participants. A significant inverse association was found between plasma levels of vitamin C and risk of diabetes, as well as between fruit and vegetable intake and DMT2 risk [43]. However,

 **DOI** 10.5455/oams.070314.rv.014

some randomized, crossover, double-blind intervention trials reported no benefit effects after supplementation of vitamin C (3000 mg/day) for 2 weeks in DMT2 subjects or 800 mg/day for 4 weeks, respectively [44 45]. In contrast, it has been found benefit of 1000 mg/day of vitamin C for 4 months [46], as well as the reduced red blood cell sorbitol/plasma glucose ratio after 2 weeks of the same doses [47].

Several investigations showed a positive effect of vitamin C on a cardiovascular system in DMT1 patients [19]. Also, a combination of vitamins C and E in these subjects improves renal function [4]. Some other studies showed that simultaneous treatment with both vitamins C and E has a positive effect on the cardiovascular system in DMT1 patients, but a negative effect in subjects with DMT2 [48, 49]. The studies focused on the use of vitamin E, unfortunately, do not provide sufficient evidence that the vitamin E reaches the target cells [38]. Furthermore, it has been shown that the α-lipoic acid enhances the function of the nerve and gives better results in the treatment of DM compared to vitamin E. Also, the vitamin C was not able to provide greater protection from the occurrence of cardiovascular complications, in comparison with the α-lipoic acid which use in the prevention of cardiovascular complications is considering [38]. However, the results of the vitamins should not be generalized for all antioxidants. Treatment with vitamins, as a class of compounds with the expected effects, ignores a wide range of their chemical and pharmacological properties [50].

Vitamin D and calcium homeostasis have also been reported to be associated to DM [51, 52]. The presence of vitamin D receptors (VDR) in the pancreatic β-islet cells support the role of vitamin D in subjects with DMT2 [53]. Furthermore, reduced overall risk of the disease in subjects who ingest 800 IU/day of vitamin D has been reported [51, 54]. In this context, data from the Women's Health Study showed that among women taking more than 511 IU/day of vitamin D reduced the risk of DMT2 when compared to ingesting 159 IU/day [55].

Therefore, clinical trials with antioxidants in DM are limited and focused majority on the use of vitamins E and C, and in recent years, α-lipoic acid [19, 56]. In any case, choice and dosage of the used antioxidant is very important [38] and it is recommended that a high dose of antioxidants should not be given as monotherapy, but in combination with other antidiabetic drugs, due to the possibilities of disruption of antioxidant/pro-oxidant balance [57, 58]. Hopefully, further studies related to the pathophysiology of OxS and to the role of antioxidants in the treatment of DM, will lead for sure to a number of clinical trials which will confirm therapeutic effects of antioxidants.

Several cross-sectional and interventional studies reported that dietary intake of micronutrients can lead to reduced levels of OxS, proinflammatory cytokines, and could be a risk for DMT2 [43, 49, 59]. Such an approach may represent a novel strategy for the prevention of DMT2. In this context, some researchers have investigated the dietary antioxidants from plant food materials [60], and they found a strong antioxidative activity in curcuminoids, the main yellow pigments in *Curcuma longa* (turmeric), known to possess antioxidant activity many years ago [61]. Dietary antioxidants possess the direct scavenging activity of ROS and also induction of antioxidative enzymes including detoxification enzymes and may prevent or delay diabetes complications including renal and neural dysfunction [60].

Also, supplementation with *Allium sativum* (garlic), *Panax quinquefolius* (American ginseng), and *Panax ginseng* (Asian ginseng), with antioxidant, anti-inflammatory, and adaptogenic properties, were reported to down-regulate the OxS and the synthesis of pro-inflammatory cytokines [62]. In this context, Rashid *et al* [21] recently reviewed the therapeutic effects of naturally occurring antioxidants. An antioxidative action of microelements such as zinc (Zn) has been reported too [63, 64]. Moghaddam *et al* [65], although on small number of subjects, show that exercise training might contribute to an improvement of the antioxidative defense against ROS in men suffering from non-insulin-dependent DMT2. Even more, Venkatasamy *et al* [66] reported that multiple mechanisms through which physical exercise acts, including up-regulating mechanisms governing physiological anti-oxidant generation.

In animals, many drugs that are already used in the treatment of DM have antioxidant properties, in addition to their primary pharmacological activity (*e.g.* N-acetylcysteine) [67] and those antioxidant properties may be a key factor in the effectiveness of these drugs [67-69]. In addition, the treatment of diabetic rats with vitamins C, E and beta-carotene results in a significant reduction in the level of TBARS and GSH-Px activity, an increase in SOD activity, while CAT activity does not change. Vitamins C and E, lower the level of TBARS and GSH-Px, while the activity of CAT and SOD were increased [70]. On the other hand, numerous studies have demonstrated a reduced SOD activity [34, 70], other studies show increased [23] or there was no change in the activity of this enzyme [71]. Of interest, the reduced SOD activity in the heart of diabetic rats is normalized by α-lipoic acid; in addition, an intraperitoneal application of α-lipoic acid in diabetic rats, normalized the level of TBARS in plasma, liver and pancreas [56]. Earlier, it was observed that the reduction of the activity of GST does not change with the α-lipoic acid [72]. However, other

studies showed an increase of GSH-Px activity in the aorta in diabetic rats, which was normalized by treatment with α-lipoic acid [56, 73]. A recent *in vitro* study showed that antioxidant treatment attenuated high glucose induced increased OxS in primary rat pancreatic stellate cells (PSC) [74], a potential underlying mechanism of islet fibrosis, which may contribute to progressive β-cell failure in DMT2. Earlier, it was reported that antioxidants reduced fibrosis and α-smooth muscle actin, the most commonly used index of PSC activation, expression in the islets in Otsuka Long-Evans Tokushima Fatty rats [75].

## CONCLUDING REMARKS

Based on previous studies of OxS in DM, today it became clear that OxS is a cause but also at the same time a consequence in both genesis and pathogenesis of DM. It could be concluded that OxS in DM occurs at the initial stage of disease, and progressively increases with the development of disorders in DM, wherein the antioxidative system (AOS) more exhausts. Therefore, it is necessary to maintain functional AOS, in terms of both prevention and therapy of DM. Further studies are needed to elucidate either direct or indirect antioxidative effects of drugs currently used in the treatment of DM and also to differentiate such effects from those on glycemia. Depletion of individual components of AOS during DM, suggesting an increased intake of antioxidants necessary for certain compensation, as well as to prevent and/or mitigate the occurrence of pathological changes caused by insufficient antioxidant protection [35, 76]. When application of antioxidants is planned in the treatment of DM, special attention should be paid to the applying dose of chosen antioxidants, in both cases as monotherapy and in combination with the main drugs used in the treatment of DM. Applying nutritional intervention to decrease OxS, as well as inflammation, might represent the first-line strategy for prevention and treatment of DMT2, including lifestyle change and exercise [77].

## ACKNOWLEDGEMENTS

This work was supported by the project number 173033 funded by the Ministry of Science and Environmental Protection.

## COMPETING INTERESTS

The authors declare that they have no conflict of interest.

## REFERENCES

1. Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol 1997; 82:291-5.
2. Bae YS, Oh H, Rhee SG, Yoo YD. Regulation of reactive oxygen species generation in cell signaling. Mol Cells 2011; 32:491-509.
3. Ceriello A. Hyperglycaemia and the vessel wall: the pathophysiological aspects on the atherosclerotic burden in patients with diabetes. Eur J Cardiovasc Prev Rehabil 2010; 17:S15-9.
4. Gaede P, Poulsen HE, Parving HH, Pedersen O. Double-blind, randomised study of the effect of combined treatment with vitamin C and E on albuminuria in Type 2 diabetic patients. Diabet Med 2001; 18:756-60.
5. Wang X, Li YL, Wu H, Liu JZ, Hu JX, Liao N, Peng J, Cao PP, Liang X, Hai CX. Antidiabetic effect of oleanolic acid: a promising use of a traditional pharmacological agent. Phytother Res 2011; 25:1031-40.
6. Choi SW, Benzie IF, Ma SW, Strain JJ, Hannigan BM. Acute hyperglycemia and oxidative stress: direct cause and effect? Free Radic Biol Med 2008; 44:1217-31.
7. Porte D, Jr. Clinical importance of insulin secretion and its interaction with insulin resistance in the treatment of type 2 diabetes mellitus and its complications. Diabetes Metab Res Rev 2001; 17:181-8.
8. Kashiwagi A, Asahina T, Nishio Y, Ikebuchi M, Tanaka Y, Kikkawa R, Shigeta Y. Glycation, oxidative stress, and scavenger activity: glucose metabolism and radical scavenger dysfunction in endothelial cells. Diabetes 1996; 45:S84-6.
9. Huebschmann AG, Regensteiner JG, Vlassara H, Reusch JE. Diabetes and advanced glycoxidation end products. Diabetes Care 2006; 29:1420-32.
10. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001; 414:813-20.
11. Lazalde-Ramos BP, Zamora-Perez AL, Sosa-Macias M, Guerrero-Velazquez C, Zuniga-Gonzalez GM. DNA and oxidative damages decrease after ingestion of folic acid in patients with type 2 diabetes. Arch Med Res 2012; 43:476-81.
12. Halliwell B, Gutteridge JM. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 1984; 219:1-14.
13. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. Trends Immunol 2004; 25:4-7.
14. Gumieniczek A, Krzywdzinska M, Nowak M. Modulation of nitrosative/oxidative stress in the lung of hyperglycemic rabbits by two antidiabetics, pioglitazone and repaglinide. Exp Lung Res 2009; 35:371-9.
15. Adameova A, Xu YJ, Duhamel TA, Tappia PS, Shan L, Dhalla NS. Anti-atherosclerotic molecules targeting oxidative stress and inflammation. Curr Pharm Des 2009; 15:3094-107.
16. Nikolic D, Katsiki N, Montalto G, Isenovic ER, Mikhailidis DP, Rizzo M. Lipoprotein subfractions in metabolic syndrome and obesity: clinical significance and therapeutic approaches. Nutrients 2013; 5:928-48.
17. Ceriello A, Lush CW, Darsow T, Piconi L, Corgnali M, Nanayakkara N, Frias JP, Maggs D. Pramlintide reduced markers of oxidative stress in the postprandial period in patients with type 2 diabetes. Diabetes Metab Res Rev 2008; 24:103-8.
18. Maiese K. Diabetic stress: new triumphs and challenges to maintain vascular longevity. Expert Rev Cardiovasc Ther 2008; 6:281-4.

 **DOI** 10.5455/oams.070314.rv.014

**19.** Haidara MA, Yassin HZ, Zakula Z, Mikhailidis DP, Isenovic ER. Diabetes and antioxidants: myth or reality? Curr Vasc Pharmacol 2010; 8:661-72.

**20.** Gupta S, Gambhir JK, Kalra O, Gautam A, Shukla K, Mehndiratta M, Agarwal S, Shukla R. Association of biomarkers of inflammation and oxidative stress with the risk of chronic kidney disease in Type 2 diabetes mellitus in North Indian population. J Diabetes Complications 2013; 27:548-52.

**21.** Rashid K, Sinha K, Sil PC. An update on oxidative stress-mediated organ pathophysiology. Food Chem Toxicol 2013; 62:584-600.

**22.** Isenovic ER, Divald A, Milivojevic N, Grgurevic T, Fisher SE, Sowers JR. Interactive effects of insulin-like growth factor-1 and beta-estradiol on endothelial nitric oxide synthase activity in rat aortic endothelial cells. Metabolism 2003; 52:482-7.

**23.** Aladag I, Eyibilen A, Guven M, Atis O, Erkokmaz U. Role of oxidative stress in hearing impairment in patients with type two diabetes mellitus. J Laryngol Otol 2009; 123:957-63.

**24.** Vikram DS, Rivera BK, Kuppusamy P. *In vivo* imaging of free radicals and oxygen. Methods Mol Biol 2010; 610:3-27.

**25.** Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 2005; 54:1615-25.

**26.** Calder PC, Albers R, Antoine JM, Blum S, Bourdet-Sicard R, Ferns GA, Folkerts G, Friedmann PS, Frost GS, Guarner F, Lovik M, Macfarlane S, Meyer PD, M'Rabet L, Serafini M, van Eden W, van Loo J, Vas Dias W, Vidry S, Winklhofer-Roob BM, Zhao J. Inflammatory disease processes and interactions with nutrition. Br J Nutr 2009; 101:S1-45.

**27.** Ceriello A, Testa R. Antioxidant anti-inflammatory treatment in type 2 diabetes. Diabetes Care 2009; 32:S232-6.

**28.** Rask-Madsen C, King GL. Proatherosclerotic mechanisms involving protein kinase C in diabetes and insulin resistance. Arterioscler Thromb Vasc Biol 2005; 25:487-96.

**29.** Browning LM, Jebb SA. Nutritional influences on inflammation and type 2 diabetes risk. Diabetes Technol Ther 2006; 8:45-54.

**30.** Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res 2010; 107:1058-70.

**31.** Bandeira SdeM, da Fonseca LJ, Guedes GdaS, Rabelo LA, Goulart MO, Vasconcelos SM. Oxidative stress as an underlying contributor in the development of chronic complications in diabetes mellitus. Int J Mol Sci 2013; 14:3265-84.

**32.** Kim CH. Expression of extracellular superoxide dismutase protein in diabetes. Arch Plast Surg 2013; 40:517-21.

**33.** Kimura F, Hasegawa G, Obayashi H, Adachi T, Hara H, Ohta M, Fukui M, Kitagawa Y, Park H, Nakamura N, Nakano K, Yoshikawa T. Serum extracellular superoxide dismutase in patients with type 2 diabetes: relationship to the development of micro- and macrovascular complications. Diabetes Care 2003; 26:1246-50.

**34.** Webb CB, Falkowski L. Oxidative stress and innate immunity in feline patients with diabetes mellitus: the role of nutrition. J Feline Med Surg 2009; 11:271-6.

**35.** Jozanov-Stankov O, Djuric J, Dobutovic B, Isenovic ER. Determination of total antioxidant status (TAS) as a biochemical parameter in control of workers' health. Arch Biol Sci 2009; 61:375-82.

**36.** Sasaki S, Inoguchi T. The role of oxidative stress in the pathogenesis of diabetic vascular complications. Diabetes Metab J 2012; 36:255-61.

**37.** Inoguchi T, Sasaki S, Kobayashi K, Takayanagi R, Yamada T. Relationship between Gilbert syndrome and prevalence of vascular complications in patients with diabetes. JAMA 2007; 298:1398-400.

**38.** Johansen JS, Harris AK, Rychly DJ, Ergul A. Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. Cardiovasc Diabetol 2005; 4:5.

**39.** Mayer-Davis EJ, Costacou T, King I, Zaccaro DJ, Bell RA, Insulin R, Atherosclerosis S. Plasma and dietary vitamin E in relation to incidence of type 2 diabetes: The Insulin Resistance and Atherosclerosis Study (IRAS). Diabetes Care 2002; 25:2172-7.

**40.** Ward NC, Wu JH, Clarke MW, Puddey IB, Burke V, Croft KD, Hodgson JM. The effect of vitamin E on blood pressure in individuals with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. J Hypertens 2007; 25:227-34.

**41.** Wu JH, Ward NC, Indrawan AP, Almeida CA, Hodgson JM, Proudfoot JM, Puddey IB, Croft KD. Effects of alpha-tocopherol and mixed tocopherol supplementation on markers of oxidative stress and inflammation in type 2 diabetes. Clin Chem 2007; 53:511-9.

**42.** Liu S, Lee IM, Song Y, Van Denburgh M, Cook NR, Manson JE, Buring JE. Vitamin E and risk of type 2 diabetes in the women's health study randomized controlled trial. Diabetes 2006; 55:2856-62.

**43.** Harding AH, Wareham NJ, Bingham SA, Khaw K, Luben R, Welch A, Forouhi NG. Plasma vitamin C level, fruit and vegetable consumption, and the risk of new-onset type 2 diabetes mellitus: the European prospective investigation of cancer--Norfolk prospective study. Arch Intern Med 2008; 168:1493-9.

**44.** Lu Q, Bjorkhem I, Wretlind B, Diczfalusy U, Henriksson P, Freyschuss A. Effect of ascorbic acid on microcirculation in patients with Type II diabetes: a randomized placebo-controlled cross-over study. Clin Sci (Lond) 2005; 108:507-13.

**45.** Chen H, Karne RJ, Hall G, Campia U, Panza JA, Cannon RO, 3rd, Wang Y, Katz A, Levine M, Quon MJ. High-dose oral vitamin C partially replenishes vitamin C levels in patients with Type 2 diabetes and low vitamin C levels but does not improve endothelial dysfunction or insulin resistance. Am J Physiol Heart Circ Physiol 2006; 290:H137-45.

**46.** Paolisso G, Balbi V, Volpe C, Varricchio G, Gambardella A, Saccomanno F, Ammendola S, Varricchio M, D'Onofrio F. Metabolic benefits deriving from chronic vitamin C supplementation in aged non-insulin dependent diabetics. J Am Coll Nutr 1995; 14:387-92.

**47.** Wang H, Zhang ZB, Wen RR, Chen JW. Experimental and clinical studies on the reduction of erythrocyte sorbitol-glucose ratios by ascorbic acid in diabetes mellitus. Diabetes Res Clin Pract 1995; 28:1-8.

**48.** Beckman JA, Goldfine AB, Gordon MB, Garrett LA, Keaney JF, Jr., Creager MA. Oral antioxidant therapy improves endothelial function in Type 1 but not Type 2 diabetes mellitus. Am J Physiol Heart Circ Physiol 2003; 285:H2392-8.

**49.** Rizzo MR, Abbatecola AM, Barbieri M, Vietri MT, Cioffi M, Grella R, Molinari A, Forsey R, Powell J, Paolisso G. Evidence for anti-inflammatory effects of combined administration of vitamin E and C in older persons with impaired fasting glucose: impact on insulin action. J Am Coll Nutr 2008; 27:505-11.

**50.** Gutteridge JM, Halliwell B. Antioxidants: Molecules, medicines, and myths. Biochem Biophys Res Commun 2010; 393:561-4.

**51.** Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J Clin Endocrinol Metab 2007; 92:2017-29.

**52.** Teegarden D, Donkin SS. Vitamin D: emerging new roles in insulin sensitivity. Nutr Res Rev 2009; 22:82-92.

**53.** Holick MF. Diabetes and the vitamin d connection. Curr Diab Rep 2008; 8:393-8.

**54.** Pittas AG, Dawson-Hughes B, Li T, Van Dam RM, Willett WC, Manson JE, Hu FB. Vitamin D and calcium intake in relation to type 2 diabetes in women. Diabetes Care 2006; 29:650-6.

**55.** Liu S, Song Y, Ford ES, Manson JE, Buring JE, Ridker PM. Dietary calcium, vitamin D, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. Diabetes Care 2005; 28:2926-32.

**56.** Bitar MS, Ayed AK, Abdel-Halim SM, Isenovic ER, Al-Mulla F. Inflammation and apoptosis in aortic tissues of aged type II diabetes: amelioration with alpha-lipoic acid through phosphatidylinositol 3-kinase/Akt- dependent mechanism. Life Sci 2010; 86:844-53.

**57.** Haidara M, Mikhailidis DP, Yassin HZ, Dobutovic B, Smiljanic KT, Soskic S, Mousa SA, Rizzo M, Isenovic ER. Evaluation of the possible contribution of antioxidants administration in metabolic syndrome. Curr Pharm Des 2011; 17:3699-712.

**58.** Opara EC. Oxidative stress, micronutrients, diabetes mellitus and its complications. J R Soc Promot Health 2002; 122:28-34.

**59.** Bartlett HE, Eperjesi F. Nutritional supplementation for type 2 diabetes: a systematic review. Ophthalmic Physiol Opt 2008; 28:503-23.

**60.** Osawa T, Kato Y. Protective role of antioxidative food factors in oxidative stress caused by hyperglycemia. Ann N Y Acad Sci 2005; 1043:440-51.

**61.** Sugiyama Y, Kawakishi S, Osawa T. Involvement of the beta-diketone moiety in the antioxidative mechanism of tetrahydrocurcumin. Biochem Pharmacol 1996; 52:519-25.

**62.** Weisberg SP, Leibel R, Tortoriello DV. Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabesity. Endocrinology 2008; 149:3549-58.

**63.** Hashemipour M, Kelishadi R, Shapouri J, Sarrafzadegan N, Amini M, Tavakoli N, Movahedian-Attar A, Mirmoghtadaee P, Poursafa P. Effect of zinc supplementation on insulin resistance and components of the metabolic syndrome in prepubertal obese children. Hormones (Athens) 2009; 8:279-85.

**64.** Prasad AS. Zinc in human health: effect of zinc on immune cells. Mol Med 2008; 14:353-7.

**65.** Moghaddam DA, Heber A, Capin D, Kreutz T, Opitz D, Lenzen E, Bloch W, Brixius K, Brinkmann C. Training increases peroxiredoxin 2 contents in the erythrocytes of overweight/obese men suffering from type 2 diabetes. Wien Med Wochenschr 2011; 161:511-8.

**66.** Venkatasamy VV, Pericherla S, Manthuruthil S, Mishra S, Hanno R. Effect of physical activity on insulin resistance, inflammation and oxidative stress in diabetes mellitus. J Clin Diagn Res 2013; 7:1764-6.

**67.** Kamboj SS, Sandhir R. Protective effect of N-acetylcysteine supplementation on mitochondrial oxidative stress and mitochondrial enzymes in cerebral cortex of streptozotocin-treated diabetic rats. Mitochondrion 2011; 11:214-22.

**68.** Prior SL, Gable DR, Cooper JA, Bain SC, Hurel SJ, Humphries SE, Stephens JW. Association between the adiponectin promoter rs266729 gene variant and oxidative stress in patients with diabetes mellitus. Eur Heart J 2009; 30:1263-9.

**69.** Robertson RP. Antioxidant drugs for treating beta-cell oxidative stress in type 2 diabetes: glucose-centric versus insulin-centric therapy. Discov Med 2010; 9:132-7.

**70.** Kedziora-Kornatowska K, Szram S, Kornatowski T, Szadujkis-Szadurski L, Kedziora J, Bartosz G. Effect of vitamin E and vitamin C supplementation on antioxidative state and renal glomerular basement membrane thickness in diabetic kidney. Nephron Exp Nephrol 2003; 95:e134-43.

**71.** Maritim AC, Sanders RA, Watkins JB 3rd. Effects of alpha-lipoic acid on biomarkers of oxidative stress in streptozotocin-induced diabetic rats. J Nutr Biochem 2003; 14:288-94.

**72.** Obrosova IG, Fathallah L, Greene DA. Early changes in lipid peroxidation and antioxidative defense in diabetic rat retina: effect of DL-alpha-lipoic acid. Eur J Pharmacol 2000; 398:139-46.

**73.** Kocak G, Aktan F, Canbolat O, Ozogul C, Elbeg S, Yildizoglu-Ari N, Karasu C. Alpha-lipoic acid treatment ameliorates metabolic parameters, blood pressure, vascular reactivity and morphology of vessels already damaged by streptozotocin-diabetes. Diabetes Nutr Metab 2000; 13:308-18.

**74.** Ryu GR, Lee E, Chun HJ, Yoon KH, Ko SH, Ahn YB, Song KH. Oxidative stress plays a role in high glucose-induced activation of pancreatic stellate cells. Biochem Biophys Res Commun 2013; 439:258-63.

**75.** Lee E, Ryu GR, Ko SH, Ahn YB, Yoon KH, Ha H, Song KH. Antioxidant treatment may protect pancreatic beta cells through the attenuation of islet fibrosis in an animal model of type 2 diabetes. Biochem Biophys Res Commun 2011; 414:397-402.

**76.** Martini LA, Catania AS, Ferreira SR. Role of vitamins and minerals in prevention and management of type 2 diabetes mellitus. Nutr Rev 2010; 68:341-54.

**77.** Garcia-Bailo B, El-Sohemy A, Haddad PS, Arora P, Benzaied F, Karmali M, Badawi A. Vitamins D, C, and E in the prevention of type 2 diabetes mellitus: modulation of inflammation and oxidative stress. Biologics 2011; 5:7-19.



 **DOI** 10.5455/oams.070314.rv.014