eISSN 2255-0569

ORIGINAL

Status of non-enzymatic antioxidant vitamins (C and E) in patients either with type 2 diabete mellitus or hypertension alone and coexisted diabetes and hypertension

Estado de las vitaminas antioxidantes no enzimáticas (C y E) en pacientes con diabetes mellitus de tipo 2 o con hipertensión sola y con diabetes e hipertensión coexistentes

Shaheenkousar Hattiwale[1], Jameel J Jargar[2], Haroon Rashid Hattiwale[1], Mohammad Muzammil Ahmed[1], Mohammed Nazeer[1]

1. Department of Basic Medical Sciences, College of Medicine 11952, Majmaah University, Al Majmaah, Kingdom of Saudi Arabia.
2. Department of Basic Medical Sciences, Al- Baha University, Al Baha, Kingdom of Saudi Arabia.

Corresponding author
Dr. Shaheenkousar Hattiwale, MD, PhD
E-mail: sh.kousar@mu.edu.sa / shaheenmaniyar@yahoo.com

Received: 19 - IV - 2022
Accepted: 30 - IV - 2022

doi: 10.3306/AJHS.2022.37.04.47

Abstract

Aim: Oxidative stress is implicated as cause and consequence of both hypertension and diabetes. Antioxidant vitamins play a vital role in maintaining redox balance both in health and disease. Therefore we have estimated and compared the level of non-enzymatic antioxidant vitamin C and vitamin E status in healthy control subjects,T2DM, hypertensive patients and patients with coexisted diabetes and hypertension.

Methods: 30-40 years old male volunteered were divided in 4 groups (n=30 in each group); group I: Healthy controls, group II: T2DM patients, group III: Hypertensive patients and group IV: Patients with co-existed type 2 diabetes and hypertension. The anthropometric parameters, blood pressure, fasting and postparendial blood sugar, serum lipid peroxide, vitamin C and E were evaluated in this study.

Results: In our study the mean concentration of serum vitamin C and E in T2DM and hypertensive patients and patients with coexisted T2DM and hypertension was found to be significantly lower and serum lipid peroxide was higher than healthy control subjects (p <0.05).

Coclusion: The oxidative stress in T2DM and hypertensive male patients is reflected by lower serum concentration of antioxidant vitamin C and E as compared to non-diabetic, normotensive healthy male counterparts. In addition, the percentage decrease of these antioxidant vitamins was more in patients with coexisted T2DM with hypertension compared to T2DM or hypertension alone. Early onset type 2 diabetes mellitus and hypertension in young age has more adverse impact on cardiovascular health. Hence supplementation of vitamin C and E may be considered as an adjuvant therapy in the management of T2DM and hypertension and also to prevent premature cardiovascular related complications in young patients.

Key words: Vitamin C, vitamin E, oxidative stress, Type 2 diabetes mellitus, hypertension, coexisted diabetes and hypertension.

Resumen

Objetivo: El estrés oxidativo está implicado como causa y consecuencia tanto de la hipertensión como de la diabetes. Las vitaminas antioxidantes desempeñan un papel fundamental en el mantenimiento del equilibrio redox tanto en la salud como en la enfermedad. Por lo tanto, hemos estimado y comparado el nivel de vitamina C y vitamina E antioxidante no enzimática en sujetos sanos de control, DMT2, pacientes hipertensos y pacientes con diabetes e hipertensión coexistentes.

Métodos: Se dividió a los voluntarios de entre 30 y 40 años en 4 grupos (n=30 en cada grupo); grupo I: controles sanos, grupo II: pacientes con DMT2, grupo III: pacientes hipertensos y grupo IV: pacientes con diabetes tipo 2 e hipertensión coexistentes. En este estudio se evaluaron los parámetros antropométricos, la presión arterial, la glucemia en ayunas y postparto, el peróxido lipídico sérico y la vitamina C y E.

Resultados: En nuestro estudio, la concentración media de vitamina C y E en suero en los pacientes con DMT2 e hipertensos y en los pacientes con DMT2 e hipertensión coexistentes resultó ser significativamente menor y el peróxido lipídico en suero fue mayor que en los sujetos de control sanos (p <0,05).

Coclusión: El estrés oxidativo en los pacientes varones con DMT2 e hipertensión se refleja en una menor concentración sérica de vitaminas C y E antioxidantes en comparación con los varones sanos no diabéticos y normotensos. Además, el porcentaje de disminución de estas vitaminas antioxidantes fue mayor en los pacientes con DMT coexistente con hipertensión en comparación con la DMT o la hipertensión solas. La diabetes mellitus de tipo 2 y la hipertensión de aparición temprana tienen un impacto más adverso en la salud cardiovascular. Por lo tanto, la administración de suplementos de vitamina C y E puede considerarse un tratamiento coadyuvante en el tratamiento de la DMT2 y la hipertensión, así como para prevenir las complicaciones cardiovasculares prematuras en pacientes jóvenes.

Palabras clave: Vitamina C, vitamina E, estrés oxidativo, diabetes mellitus tipo 2, hipertensión, diabetes e hipertensión coexistentes.

Introduction

Diabetes and hypertension are two important lifestyle related diseases affecting around 285 million and 1 billion people, respectively around the world[1]. As a result of increased global population, prevalence of early onset of overweight/obesity and unhealthy lifestyle, these figures are expected to increase by approximately 50% over the next 20 years[2-4]. Cardiovascular diseases account for 40-80% of deaths in diabetic patients and about 12 million deaths each year; known to be one of major killer diseases in the world[5]. Hypertension is one of the most common cardiovascular diseases and it is a serious public health issue affecting more than 25% of adult population in both economically underdeveloped as well as developed countries[6]. About 85% of T2DM patients have blood pressure more than 130/80 mmHg. Understanding, why hypertension is a prevailing comorbidity among T2DM patients is a serious issue in the research community today. Hence, it would be of significant use to find out the association between these two catstrophic diseases early in their pathophysiological process in checking their major complications. Previous clinical and experimental research studies have reported that oxidative stress, through excess free radical formation, plays a significant role in the onset of T2DM and hypertension[7]. Oxidative stress causes destruction of cellular membrane lipids, has been attributed along with other types of intracellular and DNA oxidative damage in the normal senescent process and in pathogenesis of a number of chronic diseases. The integrated antioxidant mechanisms, including endogenous antioxidant enzymes and exogenous antioxidant vitamins, exist to restrict the harmful implications of oxidative stress. An inadequate intake of dietary antioxidant micronutrients may compromise redox homeostasis, thus aggravate overall oxidative stress[8]. Hence, the aim of this study is to estimate and inspect the level of non-enzymatic antioxidant vitamin C and vitamin E status in patients with T2DM, hypertension and coexisted diabetes and hypertension.

Materials and methods

Source of data

Known patients of type 2 diabetes, hypertension and co-existed type-2 diabetes with hypertension visiting Al-Ameen Medical College Hospital Vijayapur, Luqman Unani Medical Hospital Vijayapur and District Hospital Vijayapur were selected for the study.

Inclusion criteria

Control group: Healthy adult men, between 30-40 years, non-diabetic, normotensive no history or evidence of renal, cardiorespiratory and other chronic diseases. All participants were non-smokers, non-alcoholic and no history of long-term drug intake.

Patients with Type 2 diabetes: Confirmed type 2 diabetic, male, normotensive patients between 30-40 years attending the clinic for regular follow up for more than 6 months were included in this group.

Patients with hypertension: Hypertensive male patients between 30-40 years, with normal blood glucose (normoglycemic) attending the clinic for regular follow up for more than 6 months were included in this group.

Patients with co-existed Type 2 diabetes and hypertension: 30-40 years old, male patients visiting the OPD for more than six months

Exclusion Criteria

Patients with type 1 diabetes mellitus, other endocrinal disorder like Cushing's syndrome, thyroid disorder, chronic respiratory, coronary artery diseases, abnormal renal function or hepatic dysfunction were excluded from the study. Healthy men or patients below 30 years or above 40 years, smokers, alcoholic, with a history of long term multi vitamin intake were also excluded from this study.

Sample size

Total 120 cases i.e., sample size of 30 in each group was calculated by taking co-existed type- 2 diabetes and hypertension with alpha error of 0.05 and power of 90.

Study design

Group I: Healthy individuals non diabetic and normotensive (Controls)
Group II: Patients with type 2 diabetes (T2DM)
Group III: Patients with hypertension (HTN)
Group IV: Patients with co-existed type 2 diabetes and hypertension (T2DM + HTN)

Methods of collection of data

Details of the study was explained to the subjects/ patients and informed written consent was obtained from volunteers. The entire study was conducted as per the guidelines mentioned in the Declaration of Helsinki. The research protocol was approved by the institutional ethical committee.

Physical Parameters

Height was measured by using wooden stadiometer and weight was measured by using weighing scale (Tokyo, Japan) as per WHO international guidelines[9]. Body mass index (BMI) was calculated by Quetelet's index. Body surface area (BSA) was recorded by Dubois' nomogram.

Physiological Parameters

Systolic and diastolic blood pressure of each participatant was recorded in right arm in supine position using a standardized sphygmomanometer (Elko, India). An average of two recordings of both systolic and diastolic blood pressure was calculated. Hypertension (HTN) was defined following the criteria of the Joint National Committee on Prevention, Detection, Evaluation and

Treatment of High Blood Pressure (JNC 7 criteria)[10].
Biochemical Parameters

Five millilitres of blood sample was collected from each participant after overnight fast in a clean sterile plain tube and blood sample was allowed to stand at room temperature for 2 hours. The sample was centrifuged at 3000rpm for half an hour to collect serum. Postprandial plasma glucose was measured 2 hours after meal. Fasting and postprandial blood glucose levels were estimated by Auto analyser EM 360 (Transasia, ERBA Mannheim, Germany). The serum lipid peroxide was measured by the method of Satoh K[11]. Serum vitamin C was estimated by the Roe and Koether, 1943 Method[12]. Serum vitamin E (α-tocopherol) was determined by using non-antibody coated microplate with ELISA reader (ERBA-Lisa Scan II, Mannheim, Germany), Jargar et al, 2012 method[13].

Statistical Analysis

The results of each group were expressed in terms of mean + standard deviation. To determine the significance of inter-group differences, one way analysis (ANOVA) followed by 'post hoc t test' were done. A value of $p < 0.05$ was interpreted as statistically significant. The mean values of the groups were subtracted from the respective control group and the differences were calculated in terms of percent change.

Results

The clinical investigations and anthropometric parameters of group I (control), group II (type 2 diabetic patients), group III (hypertensive patients) and group IV (coexisted type 2 diabetic and hypertensive patients) are presented in **table I**.

Table I shows mean weight of group II-T2DM (82.43 + 28.13 kg) and group IV-T2DM + HTN (87.82 + 14.32 kg) patients was significantly more than healthy control men (75.12 + 23.04 kg); the mean values of BMI and BSA group II (T2DM), group III (HTN) and group IV (T2DM + HTN) patients were significantly greater than control group ($p < 0.05$). In physiological parameters the systolic and diastolic blood pressure was significantly higher in group III (HTN) and group IV (T2DM + HTN) patients than group II (T2DM) patients and group I control subjects ($p < 0.05$). The fasting and postprandial blood glucose was higher in group II (T2DM) and group IV (T2DM + HTN) patients than group III (HTN) patients and group I control subjects.

Table II shows biochemical investigation results in our study. The serum concentration of lipid peroxide in group II (T2DM), group III (HTN) and group IV (T2DM + HTN) patients was significantly more than group I (control subjects) ($p < 0.05$). The percentage increase of serum lipid peroxide in group I (control subjects) vs group II (T2DM) patients was 76.72%; group I (control subjects) vs group III (HTN) patients was 62.07% and group I (control subjects) vs group IV (T2DM + HTN) patients was highest 90.52% as depicted in **figure 1**. The serum concentration of vitamin C in group II (T2DM), group III (HTN) and group IV (T2DM + HTN) patients were significantly lower than group I control subjects ($p < 0.05$). The percentage decrease of serum vitamin C in group I (control subjects) vs group II (T2DM) patients was -42.96%; group I (control subjects) vs group III (HTN) patients was -38.8% and group I (control subjects) vs group IV (T2DM + HTN) patients was lowest -68% as shown in **figure 1**. The serum concentration of vitamin E in group II (T2DM), group III (HTN) and group IV (T2DM + HTN) patients was significantly lesser than group I (control subjects) ($p < 0.05$). The percentage decrease of serum

Table I: Clinical investigations in study groups with healthy controls.

Clinical Investigations	Group I (Controls)	Group II (T2DM)	Group III (HTN)	Group IV (T2DM + HTN)
Height (cm)	171.13 + 7.67[a]	169.46 + 5.24[a]	170.55 + 10.07[a]	172.23 + 6.21[a]
Weight (kg)	75.12 + 23.04[a]	82.43 + 28.13[b]	78.31 + 22.32[a]	87.82 + 14.32[c]
BMI (kg/m^2)	23.88 + 2.76[a]	26.04 ± 4.25[b]	26.71 ± 5.18[b]	27.37 ± 4.51[b]
BSA (m^2)	1.89 + 0.14[a]	1.96 + 0.32[b]	1.92 + 0.28[b]	2.04 + 0.19[c]
SBP (mmHg)	112.76 ± 8.21[a]	121.23 ± 11.78[b]	154.55 ± 16.98[c]	154.46 ± 19.18[c]
DBP (mmHg)	80.11 ± 10.09[a]	81.32 ± 9.97[a]	98.11 ± 12.15[b]	94.81 ± 16.09[b]
Fasting blood glucose (mg/dL)	86.94 ± 8.14[a]	146.65 ± 49.86[b]	89.97 ± 12.02[a]	143.76 ± 61.43[b]
Postprandial blood glucose (mg/dL)	110.24 ± 20.22[a]	229.42 ± 94.87[b]	116.77 ± 19.95[a]	230.04 ± 90.22[b]

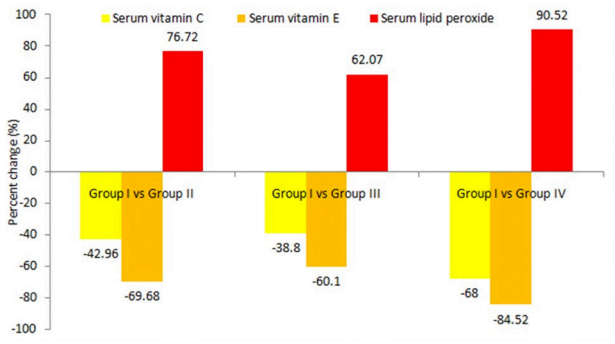
T2DM- Type 2 diabetic, HTN- Hypertensive, BMI-Body mass index, BSA-Body surface area, SBP- Systolic blood pressure, DBP- Diastolic blood pressure. Each value is mean +SD of 30 cases in each group (n=30). In each row, values with different superscripts (a, b, c, d) were significantly different from each other ($p < 0.05$). *Post-hoc* t-test analysis was used to test for differences among the means when analysis of variance (ANOVA) indicated a significant *p* value.

Table II: Serum levels of non-enzymatic antioxidant vitamins (C & E) and lipid peroxide in study groups with healthy controls.

Biochemical Investigations	Group I (Controls)	Group II (T2DM)	Group III (HTN)	Group IV (T2DM + HTN)
Serum lipid peroxide (µmol/L)	1.16 ± 0.12[a]	2.05 ± 0.21[b]	1.88 ± 0.05[c]	2.21 ± 0.22[d]
Serum vitamin C (mg/dL)	13.22 ± 2.76[a]	7.54 ± 1.92[b]	8.09 ± 2.81[b]	4.23 ± 3.06[c]
Serum vitamin E (µg/dL)	18.67 ± 1.82[a]	5.66 ± 2.20[b]	7.45 ± 1.97[c]	2.89 ± 2.14[d]

T2DM- Type 2 diabetic, HTN- Hypertensive. Each value is mean + SD of 30 cases in each group. In each row, values with different superscripts (a, b, c, d) were significantly different from each other ($p < 0.05$). *Post-hoc* t-test analysis was used to test for differences among the means when analysis of variance (ANOVA) indicated a significant *p* value.

Figure 1: Percent change graph of non-enzymatic antioxidant vitamins (C & E) and lipid peroxide in study groups verses healthy controls.



vitamin E in group I (control subjects) vs group II (T2DM) patients was -69.68%; group I (control subjects) vs group III (HTN) patients was -60.1% and group I (control subjects) vs group IV (T2DM + HTN) patients was again lowest -84.52% as shown in **figure 1**.

Discussion

This study emphasizes on the influence of non-enzymatic antioxidants like vitamin C and vitamin E on T2DM, hypertension and co-existed diabetes and hypertension and may explain the mechanism of disease pathogenesis.

The concentration of antioxidant vitamins C and E in a biological system also serve as oxidative stress biomarkers. The significant reduction of serum vitamin C and E and increase in lipid peroxide in young type 2 diabetic patients in our study indicates increase production of free radicals more than its clearance. Many other investigators have also reported that oxidative stress increases insulin resistance and plays an important role in pathogenesis of type 2 diabetes[14]. Hyperglycemia induced oxidative stress causes cellular and molecular changes like protein oxidation, lipid peroxidation and DNA damage and play a major role in progression of micro-vascular and macro-vascular complications in type 2 diabetes, causing systemic manifestations like retinopathy, nephropathy, neuropathy and accelerated coronary artery disease. The severity of tissue damage and complications are directly corelated with level/duration of hyperglycemia and consequently oxidative stress[15,16]. In research studies and meta-analysis conducted previously on type 2 diabetic male patients revealed that supplementation of vitamin C and E has improved redox balance, fasting blood glucose, lipid profile, insulin resistance, glycemic control and clinical condition of diabetic patients[17,18].

Serum lipid peroxide is well known oxidative stress biomarker, increased in hypertensive patients compared to normotensive subjects in this study suggestive of role of free radicals in pathogenesis of human hypertension. The experimental studies conducted on animal models also suggest that ROS are one of the key factors associated with endothelial dysfunction, modulators and mediators of angiotensin II induced vasoconstriction, hence play a vital role in pathogenesis of hypertension. The increased generation of free radicals in vascular smooth muscle/ endothelial cells and decreased NO bioavailability may be responsible for vascular remodeling and arterial stiffness in hypertensive patients[19,20]. In healthy individuals the vascular tissue is protected from the assault of ROS by both endogenous antioxidant enzymes and exogenous antioxidant vitamins found in vascular smooth muscles and adventitia[21]. The serum concentration of antioxidant vitamins; vitamin C and E were decreased in hypertensive patients compare to normotensive subjects in our study, probably these antioxidant vitamins were utilized for quenching of free radicals and termination of chain reactions in vascular cells to counteract oxidative stress. Previous experimental studies have showed that the supplementation of vitamin C and E has improved the plasma antioxidant status and reduced the progression of hypertension in animal model[22]. The. vitamin C and E are potent antioxidants and also believed to increases the activity of eNOS and bioavailability of nitric oxide (NO) and thereby improves endothelial dysfunction in hypertensive models[23].

In our study the oxidative stress markers were significantly more affected in patients with coexisted T2DM with hypertension than patients either with T2DM or hypertension alone as reflected by increase serum lipid peroxidation and decreased vitamin C and E concentration. The prevalence of coexisted T2DM with hypertension is around 30 to 60% of patients with T2DM at the time of diagnosis. Obesity, oxidative stress, insulin resistance, dyslipidemia and metabolic syndrome share common pathophysiological pathways for both type 2 diabetes mellitus and hypertension[24,25]. Onset T2DM and hypertension at younger age between 30-40 years as evident in our study may be adversely corelated with premature cardiovascular morbidity and mortality in their middle age[26]. The antioxidant vitamin C and E play crucial roles in maintaining redox homeostasis in cells and physiological system. Specific therapies targeting free radicals using these antioxidant vitamins in young hypertensive and T2DM patients might help to improve beta cell and vascular endothelial dysfunction; thus, may reduce the long-term complications, morbidity and mortality in T2DM and hypertensive patients. These vitamins may be considered as an adjuvant therapy for the management of diabetes and hypertension. However Long-term clinical trials and placebo/case control studies are needed to demonstrate their safety and efficacy in such patients.

Conclusion

The oxidative stress is evident in young age (30-40 years old) type 2 diabetes and hypertensive male patients as reflected by lower serum concentration of antioxidant vitamin C and E as compared to non-diabetic, normotensive healthy male counterparts. In addition, the percentage decrease of these antioxidant vitamins was more in patients with coexisted T2DM with hypertension compared to T2DM or hypertension alone. Early onset of type 2 diabetes mellitus and hypertension in young age has more adverse impact on cardiovascular health. Hence supplementation of vitamin C and E may be considered as an adjuvant therapy in the management of T2DM and hypertension and also to prevent premature cardiovascular related complications in young patients.

Author's contributions

Jameel J. Jargar designed and performed the research. Shaheenkousar Hattiwale analyzed the data and prepared final manuscript. Haroon Rashid Hattiwale Mohammad Muzammil Ahmed and Mohammed Nazeer crtically revised the manuscript and approved final version.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding/support

Non funded

References

1. World Health Organization. Global status report on non-communicable diseases 2014. World Health Organization; 2014.

2. Schofield D, Shrestha RN, Cunich MM, Passey ME, Veerman L, Tanton R, et al. The costs of diabetes among Australians aged 45-64 years from 2015 to 2030: projections of lost productive life years (PLYs), lost personal income, lost taxation revenue, extra welfare payments and lost gross domestic product from Health & Wealth MOD2030. BMJ open 2017;7(1):e013158.

3. Vinothiya K, Ashokkumar N. Modulatory effect of vanillic acid on antioxidant status in high fat diet-induced changes in diabetic hypertensive rats. Biomed Pharmacotherap 2017;87:640-52.

4. Seravalle G, Grassi G. Sympathetic nervous system, hypertension, obesity and metabolic syndrome. High Blood Pressure & Cardiovascular Prevention 2016;23(3):175-9.

5. Rossi A, Zoppini G, Benfari G, Geremia G, Bonapace S, Bonora E, et al. Mitral Regurgitation and Increased Risk of All-Cause and Cardiovascular Mortality in Patients with Type 2 Diabetes. American J Med 2017;130(1):70-6.

6. Oh JY, Allison MA, Barrett-Connor E. Different impacts of hypertension and diabetes mellitus on all-cause and cardiovascular mortality in community-dwelling older adults: the Rancho Bernardo Study. J Hypertension 2017;35(1):55-62.

7. Rahman T, Hosen I, Islam MT, Shekhar HU. Oxidative stress and human health. Adv Biosci Biotech 2012;3(7A):997.

8. Bjørklund G, Chirumbolo S. Role of oxidative stress and antioxidants in daily nutrition and human health. Nutrition 2017;33:311-21.

9. World Health Organization, "Physical Status: The Use and Interpretation of Anthropometry. Report of the WHO Expert Committee." World Health Organization Technical Report Series, Vol. 854, Geneva, 1995, pp. 1-416.

10. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo Jr JL, et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. The JNC 7 Report. J Am Med Asso 2003;289(19):2560-72.

11. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta 1978;90:37-43.

12. Roe JH, Koether CA. The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J Biol Chem 1943;147:399-407.

13. Jargar JG, Hattiwale SH, Dhundasi SA, Das S, Das KK. A modified simple method for determination of serum α-tocopherol (Vitamin E). J Basic Clin Physiol Pharmacol 2012;23(1):45-8.

14. Asmat U, Abad, K, & Ismail K. Diabetes mellitus and oxidative stress—A concise review. Saudi pharmaceutical journal 2016; 24(5), 547-53.

15. Butkowski EG, Jelinek HF. Hyperglycaemia, oxi¬dative stress and inflammation markers. Redox Rep 2017; 22: 257-64.

16. Oguntibeju OO. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. Int J Physiol Pathophysiol Pharmacol. 2019;11(3):45-63. Published 2019 Jun 15.

17. El-Aal AA, El-Ghffar EAA, Ghali AA, Zughbur MR, Sirdah MM. The effect of vitamin C and/or E supplementations on type 2 diabetic adult males under metformin treatment: A single-blinded randomized controlled clinical trial. Diabetes Metab Syndr. 2018;12(4):483-489. doi:10.1016/j.dsx.2018.03.013

18. Balbi ME., Tonin FS., Mendes AM. et al. Antioxidant effects of vitamins in type 2 diabetes: a meta-analysis of randomized controlled trials. Diabetol Metab Syndr 10, 18 (2018). https://doi.org/10.1186/s13098-018-0318-5

19. Touyz RM, Yao G, Quinn MT, Pagano PJ, Schiffrin EL. p47phox associates with the cytoskeleton through cortactin in human vascular smooth muscle cells: role in NAD(P)H oxidase regulation by angiotensin II. Arterioscler Thromb Vasc Biol 2005; 25: 512-8.

20. Touyz, RM., Briones, AM. Reactive oxygen species and vascular biology: implications in human hypertension. Hypertens Res 34, 5-14 (2011). https://doi.org/10.1038/hr.2010.201

21. Pizzino G, Irrera N, Cucinotta M, et al. Oxidative Stress: Harms and Benefits for Human Health. Oxid Med Cell Longev. 2017;2017:8416763. doi:10.1155/2017/8416763

22. Chen X, Touyz RM, Park JB, Schiffrin EL. Antioxidant effects of vitamins C and E are associated with altered activation of vascular NADPH oxidase and superoxide dismutase in stroke-prone SHR. Hypertension. 2001;38(3 Pt 2):606-611. doi:10.1161/hy09t1.094005

23. Ulker S, McKeown PP, Bayraktutan U. Vitamins reverse endothelial dysfunction through regulation of eNOS and NAD(P)H oxidase activities. Hypertension. 2003;41(3):534-539. doi:10.1161/01.HYP.0000057421.28533.37

24. Baradaran A, Nasri H, Rafieian-Kopaei M. Oxidative stress and hypertension: Possibility of hypertension therapy with antioxidants. J Res Med Sci. 2014;19(4):358-67.

25. Pouvreau C, Dayre A, Butkowski EG, de Jong B, Jelinek HF. Inflammation and oxidative stress markers in diabetes and hypertension. J Inflamm Res. 2018;11:61-68. Published 2018 Feb 19. doi:10.2147/JIR.S148911

26. Hillier TA, Pedula KL. Complications in young adults with early-onset type 2 diabetes: losing the relative protection of youth. Diabetes care. 2003;26(11):2999-3005.