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Antioxidant Multivitamin Supplementation Does Not Impede Type-Ii Diabetes Mellitus Progression

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Abstract—
The impact of long term (chronic) consumption of antioxidants on diabetes progression. Blood was withdrawn from the 51 diabetic patients who were enrolled in the study. Immediately, clinical assessment was performed to determine the changes in glucose, HbA1c and blood pressure at 0^{th} year (commencement of the study) and the 5^{th} year. Surprisingly, there were notable changes in the mean differences between the 0^{th} year and 5^{th} year of monitoring. While fasting blood sugar (FBS) did not change significantly, there was a significant change ($P<0.001$) in postprandial blood sugar (PPBS), glycosylated hemoglobin (HbA1c) and systolic as well as diastolic blood pressure. Patients with elevated HbA1C levels (>9) showed the highest BMI (>29) during the study period. The lowering of blood pressure (systolic and diastolic) during five years may be due to administration of antihypertension medications and blood thinners. While antioxidants may be helpful to curb diabetes, our results suggest that antioxidant intake may enhance the risk for chronic complications of diabetes. Our studies showed a significant change in blood glucose levels (despite rigorous antidiabetic drug intake) and rise in the levels of advanced glycation end products (AGEs) such as HbA1c. Indiscriminate antioxidant/multivitamin supplementation/intake may not curb the complications of diabetes, but rather, may lead to escalation of diabetes progression rates.

Keywords— Diabetes Mellitus, chronic antioxidant supplementation; HbA1c, postprandial blood sugar; fasting blood sugar

INTRODUCTION

Diabetes mellitus is a pandemic metabolic disorder characterized by hyperglycemia, which is confirmed by oral glucose tolerance test (GTT) and persistent insulin resistance [1], which when left untreated, causes long-term cardiovascular problems (cardiomyopathies) and microvascular complications (neuropathy, nephropathy and diabetic retinopathy). India is a frontrunner in the global diabetes pandemic [2], with over 69.2 million diabetic people, as per a WHO survey conducted in 2015 [3]. Although the exact genetic cause for diabetes mellitus etiology may differ from person to person, the role of hyperglycemia and insulin resistance in the pathogenesis of type-II diabetes is well-known. Also, obesity and physical inactivity is known to contribute insulin resistance and progression of diabetes complications from minimal to vulnerable. The connection between diabetes and oxidative stress is murky and has been a subject of intense research and debate [4]. Reactive oxygen species/ROS [5] derived from excess glucose oxidation are known to cause the progression of diabetes and result in organ damage, leading to the long-term complications of the disease and further escalation to full-blown diabetes which is characterized by micro and macrovascular complications being the hallmark pathophysiological manifestations [6]. Vascular damage is also known to be a characteristic trait of diabetes mellitus. In most of these cases, the presence of sustained ROS (and oxidative stress) is considered to be responsible for the initiation and progression of its complications. Hence,

antioxidant supplementation has been considered to be an effective means to reduce the rate of disease progression through neutralization of the excessive free radicals and oxidants [5]. In India, antioxidant supplementation is not recommended on an as-needed basis and is rarely recommended for acute period of time. Also, patients self-medicate themselves indiscriminately with multivitamin formulations and other antioxidants, sometimes without the physician's consent. Since ROS are known to have physiological roles [7], we deem that indiscriminate and more importantly, chronic antioxidant intake without performing background investigation of the redox condition of any given patient subjects may alter the physiological redox conditions and cause antioxidant stress. We hypothesized that chronic multivitamin (considered as antioxidants) intake may not prevent (or reverse) the pathophysiological manifestations of diabetes. Hence, this study was conducted to identify the potential effects of multivitamin supplementation (beneficial/harmful effects) along with standard treatment regimen normally recommended to diabetes patients (anti-hyperglycemic and anti-hypertension drugs).

MATERIALS AND METHODS

A. Recruitment of Participants: Diabetic patients (n=51) who were consuming (prescribed & non-prescribed) antioxidants regularly in South region of India (Tamil Nadu) were followed. Samples were collected and clinical analyses were performed in Karunya Sugalaya Diabetic Care & Research Centre in Kumbakonam, Tamil Nadu. The mean age of the subjects was 54 years and the age of the patients ranged from 35-75. The distribution of subjects was almost 1:1 for males: females (25:26). Other pertinent details about the study participants are mentioned in Table-I. This study protocol in diabetic patients was approved by the Bharathidasan University Institution ethical committee (IEC; DM/2014/101/47). Informed consent was obtained from the patients and they were notified about being enrolled into this study.

B. Measurement of Fasting (FBS) and Postprandial Blood Sugar (PPBS): Assay of FBS and PPBS was performed by following the coupled assay of glucose oxidase-horseradish peroxidase procedure as given in the manufacturer's protocol (Biosystems, India). The serum samples of the patients were collected using standard clinical protocol. The glucose assay reagent was brought to room temperature before commencement of the assay. Separate reactions were performed for blank (no glucose), standard (100 mg/dL glucose) and test (for all fresh serum samples during the course of the five year period). The assay reagent contained phosphate (100 mM), phenol (5 mM), glucose oxidase (> 10 U/ml), peroxidase (>1 U/ml) and 4-aminoantipyrine (0.4 mM); when checked prior to use, the pH of the reagent was 7.5. The blank comprised of 1 ml assay reagent. The standard contained 10 µl of glucose (100 mg/dL stock), while 10 µl of serum (each from different patients) was mixed with 1 ml of the assay reagent. The tubes were mixed well and incubated for 10 minutes at room temperature (16-25°C) and the development of colour owing to formation of the red quinonimine product was followed spectrophotometrically at 500 nm using a semi-automated biochemistry analyzer.

C. Assay of HbA1c: HbA1c levels of the patients were quantified using a modified HPLC method (D-10™ HbA1c program 220-0101) as per the manufacturer's instructions (D-10 system, BioRad, USA). Whole blood samples of the patients were collected in vacuum tubes containing EDTA. The samples were allowed to reach room temperature (15-30 °C) prior to analysis. The instrument was calibrated after priming a new cartridge. A_{1c} low control, A_{1c} high control and patient samples were placed in the sample rack and the readings were recorded.

D. Statistical Analysis: All values are represented as mean ± SEM. Student's T-test was used to detect the statistical difference in each parameter between control and diabetes patients. The P-value less than 0.05 ($P < 0.05$) was considered statistically significant. All statistical analyses were performed using Graph pad prism 8.

RESULTS AND DISCUSSION

The free radical theory of ageing and disease (FRTAD) was proposed by Denham Harman in 1956 to explain the role of reactive oxygen species in the pathogenesis and pathophysiology of several human diseases. ROS is known to be produced during normal aerobic respiration and several ROS species were shown to be

responsible for macromolecular (protein, lipid and DNA) damage [8]. Ever since the FRTAD hypothesis became accepted, antioxidant therapy was proposed as a viable means to neutralize hyperglycemia-sponsored ROS [5] and to alleviate the ROS-mediated acceleration of the pathophysiology of diabetes [9]. In this study, the patients took antioxidants along with their medications for diabetes and hypertension for over five years.

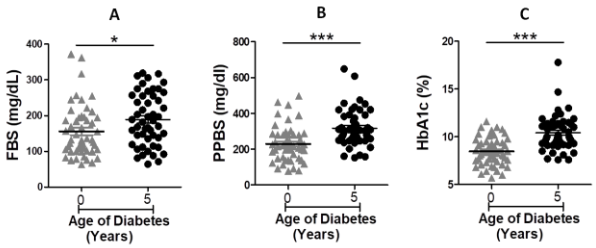

Fig. 1 Change in FBS, PPBS and HbA1c in diabetic subjects between 0th year and 5th year of study - Differences in A: Fasting blood sugar (FBS) levels, B: Postprandial blood sugar (PPBS) levels and C: Glycosylated hemoglobin in study subjects over a period of five years. Values are represented as mean±SEM. * defines P<0.05; *** defines P<0.001.

Results from the present study showed that mean fasting blood sugar level (Figure 1A) had increased significantly (P<0.05) when comparing between 0 (time of study commencement) and 5 years of diabetes progression. The significant rise in postprandial blood glucose (between 0 and 5 years) shows an increased impairment of glucose tolerance (diminished insulin action), as indicated by the elevation of mean blood sugar from ~210 to ~310 mg/dL (Figure 1 B). Indeed, antioxidants/multivitamins may not be able to reverse macromolecular damage, but might only be envisioned to prevent oxidative damage. Hence, we assessed the HbA1c levels of the patients and found that glycosylated haemoglobin levels were significantly elevated in ~50% of patients (Figure 1C), suggesting that there was a steady rise in the glucose levels (FBS/PPBS) which accelerated the formation of advanced glycation end products during the course of the study period (below 10 to above 10), despite the chronic consumption of antioxidant supplements. HbA1c is a key parameter for diagnosing diabetes and a level of >6.5 is indicative of diabetes mellitus, while a value above 10.0 signifies the progression of diabetes to an advanced stage with vulnerable complications [10]. This means that antioxidant intake was not successful in halting the progression of diabetes-mediated damage to the macromolecules (proteins/lipids etc., formation of advanced glycation end products). Therefore, we can clearly infer that chronic (and indiscriminate) antioxidant intake may not be a useful strategy to slow down the progression of diabetes, contrary to earlier findings [11, 12].

In Figure 2, the data for changes in physiological parameters such as body mass index (BMI, Figure 2A) and waist circumference (WC, Figure 2B) have been presented. Diabetes mellitus usually causes weight gain due to insulin resistance, which is triggered due to lack of physical activity and the prevalence of obesity [13]. Since insulin is antilipolytic and because there is low insulin sensitivity during diabetes mellitus, lipolysis occurs and the resulting hyperlipidemia is responsible for accentuating insulin resistance [14]. Insulin sensitivity is known to improve after weight loss. Chronic diabetes with elevated HbA1c levels (>8) is accompanied with an increase in BMI and insulin resistance. Our current line of investigation also showed that 50% patients with elevated HbA1c levels had higher BMI (>29) and those patients were prescribed with medium to high (25-40U/day) dose of insulin to control their glycaemic status (Supplemental Figure-1).

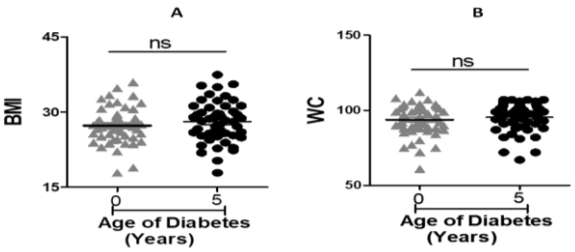

Fig. 2 Change in BMI and WC in diabetic subjects during the 5 year study period - A: Body mass index and B: waist circumference of the patients at 0 and 5 years. Values are represented as mean±SEM. Ns-non-significant.

The fact that mean BMI and WC values did not increase dramatically (except in a few cases) in the five-year period shows that the patients did not gain/lose weight. While BMI increased from mild to moderate in 29 patients, it also dropped in some patients (27 individuals); conversely, the patients' weight loss was not statistically significant. A comparison of BMI changes in the 51 patients is presented in supplementary Fig.S1. It is not clear if the antidiabetic medications had a role to play in preventing from weight gain (see Table I for the list of antidiabetic medications prescribed to the patients). The vitamin supplements taken by the patients are mentioned in Table II.

TABLE I. BASAL CHARACTERISTICS OF THE STUDY SUBJECTS

PARAMETERS	DIABETES GROUP (N=51)
Male Age (N=26)	54.19±11.06
Female Age (N=25)	54.12±10.73
Body Mass Index (BMI)	28.17±4.36
Fasting Blood Glucose (mg/dL)	192.79±93.50
Postprandial Blood Glucose (mg/dL)	327.60±118.39
HbA1c	10.13±1.75
Systolic BP	127.35±16.60
Diastolic BP	75.16±8.31

Also, since indiscriminate vitamin intake may lead to hypervitaminoses and is a risk factor for obesity [15], it may further increase insulin resistance. Indeed, indiscriminate and prolonged vitamin intake may promote cardiovascular disease and other undesirable complications during the course of diabetes progression [16].

TABLE II. DOSAGE OF ANTIOXIDANTS CONSUMED DAILY BY DIABETIC PATIENTS

MULTI-VITAMIN AND COFACTORS	PER SERVING (PER TABLET)
VITAMINS	
Vitamin A (as Acetate)	2500 IU
Vitamin E (Tocopherol)	10 IU
Vitamin K	10 mcg
Vitamin D3 (Cholecalciferol)	200 IU
Vitamin B1 (Thiamine Mononitrate)	2 mg
Vitamin B2 (Riboflavin)	3mg
Vitamin B6 (Pyridoxie Hydrochloride)	1.5mg
Niacinamide	26mg
Vitamin C (Ascorbic Acid)	50mg
Vitamin B12 (Cyanocobalamin)	1mcg
Folic Acid	0.3mg
Calcium Pantothenate	5mg
Biotin	30mcg
MINERALS:	
Zinc (as zinc sulphate Monohydrate)	15mg
Iodine (as potassium iodide)	0.15mg
Ferrous fumarate equ. To Iron	9mg
Magnesium (as Magnesium oxide)	100mg
Manganese (as Managanese sulphate monohydrate)	2.5mg
Copper (as Cupric Oxide)	2mg
Calcium (as Dibasic calcium Phosphate)	162mg
Phosphorous	125mg
Potassium (as Potassium Chloride)	40mg
Chloride	36.3mg
Chromium (as Chromium Chloride)	100mcg
Selenium (as selenium silicate)	25mcg

Nickel (Nickel sulphate)	5mcg
Silica (as colloidal silicon Dioxide)	10mcg
Vanadium	10mcg
Molybdenum (as Sodium Molybdenum)	100mcg

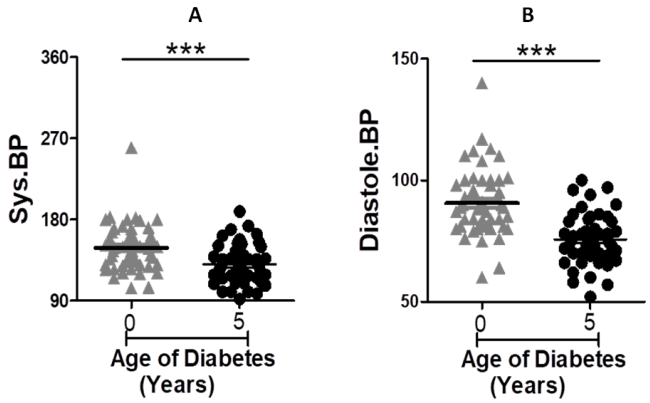

Fig. 3 Change in blood pressure (systolic and diastolic) in diabetic subjects during the study period - Blood pressure – A: systolic and B: diastolic pressure recorded at 0 and 5th years. Values are represented as mean±SEM. *** defines P<0.001.

In Figure 3A, the systolic and Figure 3B diastolic BP values of the patients are shown. We can see that the antihypertension medications have been very useful in preventing the increase of hypertension to very high levels. Diabetes is a key risk factor for hypertension. At the start of the study period (0 year), the mean level of Sys.BP was close to 160 mm Hg and diastolic BP was just under 100 mm Hg. We may infer that the antihypertension drugs had been able to keep the BP levels under control. Most of the diabetic patients (>60%) consumed anti-hypertensive drugs to maintain their blood pressure levels. However, we cannot infer any role for antioxidants in maintaining the blood pressure (or the significant reduction in the mean) during the study period. Also, the near-normal values for systolic and diastolic BP could be owing to antihypertension medication intake just prior to collection of blood samples. The very high levels of vitamin A and vitamin C (Table II) can be noted. Also, the potential contribution of minerals to the observed results is not clear. Supplemental Fig.S1 shows that BMI of most of the study subjects (n=29) increased either mildly or moderately during the 5-year period, while BMI of 22 individuals decreased.

CONCLUSION

Diabetes patients in India often self-medicate themselves with antioxidant supplements. Otherwise, they are usually administered antioxidants or antioxidant vitamin supplements by their physicians. The redox balance of cells in critical tissues such as cardiac muscle can be disturbed and this can lead to escalation of the chronic complications of diabetes mellitus and lead to undesirable microvascular changes, ultimately increasing the risk for cardiomyopathies. This study demonstrates that chronic multivitamin intake does not reduce the rate of progression of diabetes, as proved a significant increase in PPBS and HbA1c. Systolic and diastolic BP was also significantly lowered during the five year period. These results demonstrate that the lowering of mean blood pressure values may be due to BP medications. This aspect needs to be revisited and explored at the molecular level and the effect of specific antioxidant molecules in potentially inducing heart disease may need to be thoroughly investigated in the future. This is because the risk for heart disease may be higher in patients who have diabetes and also consume antioxidant supplements for a protracted time period. The prospective role of BP medication in this process ought to be explored. It is also opportune to warn that diabetes patients must not take multivitamin supplements unless and until they are necessary and advised by the physician.

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REFERENCES

1. A. D. Association, "Diagnosis and classification of diabetes mellitus," Diabetes care, vol. 33, pp. S62-S69, 2010.
2. R. Unnikrishnan, R. M. Anjana, and V. Mohan, "Diabetes mellitus and its complications in India," Nature Reviews Endocrinology, vol. 12, p. 357, 2016.
3. Y. Zheng, S. H. Ley, and F. B. Hu, "Global aetiology and epidemiology of type 2 diabetes mellitus and its complications," Nature Reviews Endocrinology, vol. 14, p. 88, 2018.
4. A. Ceriello, R. Testa, and S. Genovese, "Clinical implications of oxidative stress and potential role of natural antioxidants in diabetic vascular complications," Nutrition, Metabolism and Cardiovascular Diseases, vol. 26, pp. 285-292, 2016.
5. D. Bolignano, V. Cernaro, G. Gembillo, R. Baggetta, M. Buemi, and G. D'Arrigo, "Antioxidant agents for delaying diabetic kidney disease progression: a systematic review and meta-analysis," PLoS One, vol. 12, p. e0178699, 2017.
6. H. Kaneto, N. Katakami, M. Matsuhisa, and T.-a. Matsuoka, "Role of reactive oxygen species in the progression of type 2 diabetes and atherosclerosis," Mediators of inflammation, vol. 2010, 2010.
7. M. L. Circu and T. Y. Aw, "Reactive oxygen species, cellular redox systems, and apoptosis," Free Radical Biology and Medicine, vol. 48, pp. 749-762, 2010.
8. J. L. Evans, I. D. Goldfine, B. A. Maddux, and G. M. Grodsky, "Are oxidative stress− activated signaling pathways mediators of insulin resistance and β-cell dysfunction?," Diabetes, vol. 52, pp. 1-8, 2003.
9. J. S. Johansen, A. K. Harris, D. J. Rychly, and A. Ergul, "Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice," Cardiovascular diabetology, vol. 4, p. 5, 2005.
10. C. Bennett, M. Guo, and S. Dharmage, "HbA1c as a screening tool for detection of type 2 diabetes: a systematic review," Diabetic medicine, vol. 24, pp. 333-343, 2007.
11. J. L. Evans, B. A. Maddux, and I. D. Goldfine, "The molecular basis for oxidative stress-induced insulin resistance," Antioxidants & redox signaling, vol. 7, pp. 1040-1052, 2005.
12. J. Vertommen and I. L. De, "The effect of flavonoid treatment on the glycation and antioxidant status in Type 1 diabetic patients," Diabetes, nutrition & metabolism, vol. 12, pp. 256-263, 1999.
13. R. H. Eckel, "Insulin resistance: an adaptation for weight maintenance," The Lancet, vol. 340, pp. 1452-1453, 1992.
14. H. A. Ferris and C. R. Kahn, "Unraveling the paradox of selective insulin resistance in the liver: the brain–liver connection," Diabetes, vol. 65, pp. 1481-1483, 2016.

15.S.-S. Zhou and Y. Zhou, "Excess vitamin intake: An unrecognized risk factor for obesity," World journal of diabetes, vol. 5, p. 1, 2014.
16.M. Sheikh-Ali, J. M. Chehade, and A. D. Mooradian, "The antioxidant paradox in diabetes mellitus," American journal of therapeutics, vol. 18, pp. 266-278, 2011.