

## Impact of Physical activity on the Nutritional Biomarkers in type 2 Diabetes

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### Abstract

Diabetes mellitus is the fast spreading epidemic of the 21<sup>st</sup> century and mortality rate is high among the physically inactive middle and old aged people. In the present study the impact of physical activity on the nutritional biomarkers among young, middle and older aged diabetic subjects, living in semi urban area Thanjavur town, Tamil Nadu has been investigated. For the present study, the male subjects of 35-45, 46-55 and 56-65 years were selected. Vitamin C, E,  $\beta$  carotene and folic acid analyzed as nutritional biomarkers were found to be very low in elderly diabetic persons when compared to the physically active diabetic subjects. People with diabetes had a decline in the level of nutritional biomarkers, because of increased production of free radicals in hyperglycemia. Everyday physical activity such as walking is associated with the increased insulin sensitivity, decreased oxidative stress and enhanced antioxidant defense system.

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### Key words:

Diabetes, walking, nutritional biomarkers, vitamins, physical activity

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### INTRODUCTION

Diabetes mellitus is a progressive chronic disease emerging as a global epidemic [1]. It has a distinct pathophysiological mechanism that may lead to unique impact on eye, kidney, nerve damage and further complications besides increased risk for cardiovascular disease [2]. Further, diabetes imposes a tremendous burden on health economies mainly because of its devastating nature of complications. India is currently termed as "*diabetes capital of the world*" due to increasing number of diabetic subjects [3]. According to the diabetic atlas published in 2006 by the International Diabetes Federation, the number

of people with diabetes in India is currently around 40 million and this number is expected to rise to 70 million by 2025, unless urgent preventive steps are taken to control this disease [4]. However, it is found that despite dietary changes the main factors associated with urbanization such as obesity, physical inactivity, smoking and chronic alcohol abuse are related to the escalation of diabetes among Indians [5].

Physical inactivity is an independent risk factor for diabetes in Indians. Researchers looking at levels of physical activity among South Asians noted some awareness of its importance but a lack of putting it into practice [6]. The reasons included cultural norms, social expectations, time constraints and health problems. It has also been reported that healthcare professionals perceive South Asians as holding fatalistic beliefs surrounding their health status and are reluctant to provide lifestyle advice because of poor cultural and religious understanding[7], thus exacerbating the problem.

Simple, daily, vigorous walking can significantly improve cardiac risk factors and glucose metabolism. Because walking is accessible and relatively safe it can be easily incorporated into daily routines. It is a form of exercise that is practical and suitable for most diabetic patients, especially elderly persons.

Micronutrients are regarded as nutritional biomarkers which are used for a variety of purposes in large scale population surveys and epidemiologic studies as to provide a measure of nutritional status, in geographical area [8]. Many antioxidants such as vitamin C and carotenoids have been reported as having health protective effects. Inadequate micronutrient intakes may reduce resistance to infections and adversely affect the immune system. Therefore, there is a need to estimate the magnitude of micronutrient deficiencies and assess their consequences on health. Evidence from epidemiological studies supports the importance of an optimal micronutrient status to help in preventing chronic diseases such as coronary heart disease,

cancer, mental degeneration, diabetes and age related macular degeneration. Antioxidant micronutrients such as vitamin C, E and  $\beta$  carotene are particularly important.

Keeping the above factors in mind, Thanjavur area has been selected for the present study. It comes in the category of semi urban area. While reports are available from urban, periurban, slum areas of Chennai and Pondicherry, no study has been carried out from Thanjavur area. Recent reports have pointed out that the actual causes of death among the diabetic subjects are usage of tobacco, physical inactivity, malnutrition and excessive use of alcohol. In the present study the impact of life style intervention, physical activity on the micronutrient status among young, middle and old aged diabetic subjects, living in Thanjavur town, Tamil Nadu has been investigated.

#### MATERIALS AND METHODS

For the present study, only the male subjects were selected. The age groups selected were 35-45, 46-55 and 56-65 years and categorized as young, middle and old aged diabetic subjects. All the human volunteers were issued with a questionnaire to determine the eligibility for participation in the study. The questions elicited vital information on age, body weight, height, exercise, habits, health status, smoking habit, alcohol intake and the use of dietary supplements. Physically active diabetic subjects with the habit of walking for at least 30 min / day or 2 days once were included for the investigation. Written informed consent was obtained from all the participants of the study after providing sufficient explanation for participation in the study.

The blood sample was collected from the subjects using the method described by NCCLS [9]. The blood was collected by venous arm puncture after an overnight fasting. The puncher site was then cleaned with an antiseptic spirit and tourniquet was placed around the upper arm, i.e. 4 inches above the

intended puncture site to obstruct the return of venous blood to heart and to distend the vein. A needle was inserted into the vein and blood was collected using a syringe. During the procedure, the tourniquet was removed and samples of blood were collected into clean labeled capped tubes for estimation of various parameters. Plasma and serum were separated by centrifugation at 1300 x g for 15 min and stored at 4°C until analysis for nutritional biomarkers.

The level of plasma vitamin C was determined by the method of Omaye *et al.* [10]. Ascorbic acid is oxidized by copper to form dehydroascorbic acid and diketoglutaric acid. These products when treated with 2, 4-dinitrophenyl hydrazine (DNPH) form the derivatives bis-2, 4-dinitrophenylhydrazone which undergoes rearrangement to form a product with an absorption maximum at 520nm. Thiourea provides a mild reducing medium that helps to prevent interference from non-ascorbic acid chromogens.

Vitamin E was estimated in plasma using the method of Desai [11] based on the classical Emmerie Engle reaction. This method involves reduction of ferric ions to ferrous ions by the tocopherol and the formation of a pink colored complex with bathophenanthroline orthophosphoric acid. Absorbance of the stable chromophore is measured at 536nm.  $\beta$  Carotene was determined by the method of Bayfield and Cole [12]. Vitamin A undergoes protonation to form anhydrovitamin with strong trichloroacetic acid. The transient blue colour of the carbonium ion was measured colorimetrically at 620 nm.

The folic acid in the blood plasma was determined by enzyme linked immunosorbent assay (ELISA) method [13]. Folic Acid quantitative test is based on the principle of the ELISA. A folic acid conjugate is bound on the surface of a microtiter plate. Folic acid containing samples or standards and an antibody directed against folic acid are given into the wells of the microtiter plate. Immobilized and free folic acid competes for the antibody binding sites. After one

hour incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugate against the antibody is given into the wells and after hour incubation, the plate is washed again. Then a substrate solution is added and incubated for 20 minutes, resulting in the development of a blue colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of folic acid is indirectly proportional to the colour intensity of the test sample.

The data collected in the present study were carefully categorized, segregated and statistically analyzed. The values are expressed as Mean  $\pm$  Standard Deviation. Further, the values were analyzed by one way analysis of variance (ANOVA) using SPSS version 12.0 for windows and the levels of significances were noted.

## RESULTS

The levels of vitamin C in the diabetic subjects and physically active diabetic subjects are shown in Table 1. It can be inferred from the table that the levels of vitamin C is declined significantly ( $P < 0.01$ ) among the diabetic subjects compared to the walking diabetic subjects of different age groups.

**Table 1:** Effect of physical activity on vitamin C levels (mg/dl) among young, middle and old diabetic subjects

Groups	Age Groups	N	Mean $\pm$ SD	F-value	P Value
Normal	Control	50	1.37 $\pm$ 0.18		
	young	16	0.78 $\pm$ 0.06	189.37	0.001
	middle	20	0.69 $\pm$ 0.06		
old	14	0.59 $\pm$ 0.06			
Physically active diabetic subjects	young	16	1.27 $\pm$ 0.08	34.63	0.001
	middle	17	0.96 $\pm$ 0.08		
	old	17	0.93 $\pm$ 0.05		

Values are means  $\pm$  SD

It is revealed from Table 2 that the levels of vitamin E are significantly reduced among the elderly diabetic subjects. Walking was found to replenish the vitamin E levels among the younger diabetic subjects compared to the diabetic subjects of other age groups ( $1.42 \pm 1.03$ ;  $1.08 \pm 0.82$ ;  $1.05 \pm 0.03$  for young, middle and older diabetics respectively).

**Table 2:** Effect of physical activity on vitamin E levels (mg/dl) among young, middle and old diabetic subjects

Groups	Age Groups	N	Mean±SD	F-value	P Value
Normal	Control	50	1.30±0.22		
Diabetic subjects	young	16	1.05±0.04	43.39	0.001
	middle	20	0.96±0.11		
	old	14	0.79±0.06		
Physically active diabetic subjects	young	16	1.42±0.13	12.71	0.001
	middle	17	1.08±0.02		
	old	17	1.05±0.03		

Values are means  $\pm$  SD

Table 3 depicts the  $\beta$  carotene levels in diabetic and physically active diabetic subjects.  $\beta$  Carotene was found to be significantly depleted ( $P < 0.01$ ) among the diabetic subjects. The habit of walking was found to significantly elevate the levels of  $\beta$  carotene among younger diabetics compared to the diabetic subjects of other age groups.

**Table 3:** Effect of physical activity on beta carotene levels (mg/dl) among young, middle and old diabetic subjects

Groups	Age Groups	N	Mean±SD	F-value	P Value
Normal	Control	50	1.09±0.12		
Diabetic subjects	young	16	0.93±0.05	131.17	0.001
	middle	20	0.69±0.06		
	old	14	0.59±0.06		
Physically active diabetic subjects	young	16	1.15±0.18	8.73	0.001
	middle	17	1.02±0.05		
	old	17	0.91±0.06		

Values are means  $\pm$  SD

The levels of folic acid are shown in Table 4. Levels of folic acid was significantly reduced in the old age group. The diabetic subjects having habit of walking showed restoration of folic acid levels to near normal

among the younger diabetic subjects while the elderly diabetic subjects showed low level compared to young and middle aged diabetic subjects.

**Table 4:** Effect of physical activity on folic acid levels (mg/dl) among young, middle and old diabetic subjects

Groups	Age Groups	N	Mean±SD	F-value	P Value
Normal	Control	50	14.80±0.78		
Diabetic subjects	young	16	11.55±0.96	280.14	0.001
	middle	20	8.97±0.78		
	old	14	7.07±0.84		
Physically active diabetic subjects	young	16	13.50±1.05	141.14	0.001
	middle	17	11.00±0.82		
	old	17	9.36±0.50		

Values are means  $\pm$  SD

## DISCUSSION

Diabetes mellitus is a chronic, systemic, metabolic disease defined by hyperglycemia and characterized by alterations in the metabolism of carbohydrate, protein and lipid. Oxidative stress thought to be increased in a system where the rate of free radical production is increased and / or the antioxidant mechanism is impaired. In the present study vitamin C, E,  $\beta$  carotene and folic acid were analyzed as nutritional biomarkers in different age group of diabetic subjects with and without the habit of walking.

Pasupathi *et al.* [14] reported that people with diabetes have a low level of antioxidants because of increased production of free radicals in hyperglycemia. The antioxidants constitute the foremost defense system that limit the toxicity associated with free radicals. Our research has shown that diabetic subjects have low levels of vitamin C and vitamin E. The observed significant decrease in the level of vitamin C could be caused by increased utilization of vitamin C as an antioxidant defense against reactive oxygen species (ROS).

Vitamin E, a potent antioxidant agent, exerts a protective role as a free radical scavenger through a non enzymatic mechanism outside the cells [15]. Vitamin E also showed a similar pattern to that of

vitamin C. In elderly subjects a reduction in plasma vitamin E concentrations has been demonstrated to occur. Similarly the old diabetic subjects showed very low level of vitamin E compared to the other aged groups studied. This finding is in agreement with Esterbauer *et al.* [16] who reported that vitamin E is a lipophilic chain breaking antioxidant that scavenges lipid peroxy radicals. The decrease in vitamin E has been attributed to increased utilization for scavenging the oxyradicals generated or due to decreased vitamin C levels because there is a well established interaction between vitamin E and vitamin C.

Pasupathi *et al.* [14] stated that vitamin E is a well known physiological antioxidant and membrane stabilizer. It interrupts the chain reaction of lipid peroxidation by reacting with lipid peroxy radicals, thus protecting cell structure against damage. The decreased level of vitamin E observed in the diabetic patients is compatible with the hypothesis that plasma vitamin excess plays a protective role against increased peroxidation in diabetes.

The study of  $\beta$  carotene in the young, middle and aged group of diabetic subjects compared to physically active diabetic subjects showed that almost old aged diabetic subjects have low value of  $\beta$  carotene. Ford *et al.* [17] reported that  $\beta$  carotene is a chain breaking antioxidant and a precursor of vitamin A. Further it is also showed that plasma concentrations of vitamin A and its carrier proteins, retinol binding protein and transthyretin have decreased in diabetic patients. The underlying cause for decreased availability of the vitamin in diabetes is not clearly understood. It appears that the increased hepatic store of vitamin A is attributed to a decreased availability of its carrier proteins particularly  $\beta$  carotene. But Pasupathi *et al.* [14] stated that  $\beta$  carotene is a unique lipid soluble antioxidant, which traps peroxy radicals. Decreased level of  $\beta$  carotene among diabetic subjects is associated with increased oxidative stress and a reduced supply of circulating antioxidants in the body, which may be due to the

creation of an extra demand for antioxidants through oxidative stress.

Folic acid is regarded as a nutritional biomarker. In the present study it showed low levels in the blood of diabetic subjects particularly in the aged people. This indicated the vital link of diabetes with the folic acid which may lead to anemia. In South Asian households, prolonged cooking of vegetables is a common practice, which may destroy nearly 90% of the folate content; henceforth the protective effect of the folate is decreased [18]. Antioxidant defense mechanisms are important for protection of cells and tissues from oxidative damage which consist of non enzymic antioxidants particularly folic acid. In the present study physical activity was found to improve the folic acid level in diabetic subjects.

In the present observation the results indicated that all the nutritional biomarkers were at significantly low level in the aged people only. This may be due to the fact attributed that aging decrease the antioxidant capacity, which leads to oxidative stress. The factor that contributes to aging and the development among other diseases of diabetes mellitus is ROS. Even in later ages, lifestyle factors with brisk walking are associated with markedly lower incidence of new onset diabetes mellitus as suggested by Mozaffarian *et al.* [19].

In the present study, physical activity improved the levels of nutritional biomarkers among the walking diabetics which may be associated with the increased insulin sensitivity and decreased oxidative stress. Regular physical exercise enhances the antioxidant defense system. Physical activity is now recognized as a major component of type 2 diabetes prevention. Cohort studies have documented the lower risk of incident diabetes even for everyday activities such as walking. The entry of glucose into skeletal muscle is increased during exercise in the absence of insulin by causing an insulin independent increase in the number of GLUT-4 transporters in muscle cell membrane, increase in glucose entry persists for several hours after exercise and regular exercise

training can produce prolonged increase in insulin sensitivity [20]. Physical activity can influence insulin sensitivity in many ways including enhancing GLUT4 dependent glucose transport in skeletal muscle, increasing skeletal muscle vascularization, mitochondrial neobiogenesis and eventually tissue mass, repartitioning intracellular fat, thereby improving its utilization and fat mass loss.

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