

## Effect of Yoga on Oxidative Stress in Elderly Type 2 Diabetes Subjects

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### Abstract

**Introduction:** Aging along with increased oxidative stress has been suggested as contributory factors for initiation and progression of complications in diabetes mellitus. Present study aims to evaluate the effect of 3 month yoga on oxidative stress, BMI, blood pressure and glycaemic status in elderly type 2 diabetes patients.

**Methods:** Yoga was delivered to elderly diabetic (age>60, n=42) and younger diabetes subjects (age<60, n=45) for 3 months. Both groups continued their normal medication and diet.

**Results:** Malondialdehyde showed a positive correlation with age in participants. Antioxidants showed a negative correlation with age and malondialdehyde. Yoga resulted in significant reduction in malondialdehyde, BMI, blood pressure, fasting glucose and improvement in glutathione levels in both groups. Vitamin C and glycosylated haemoglobin showed improvement in younger subjects.

**Conclusion:** Oxidative stress is higher in elderly type 2 diabetes patients when compared to younger patients. Yoga may be beneficial for reducing oxidative stress, BMI and blood pressure in diabetes subjects irrespective of age. Yoga can be an aid for management of diabetes in improving the antioxidant potential which deteriorates with age. Glycemic status improved in younger diabetes subjects.

**Keywords:** Yoga; Elderly type 2 diabetes; Oxidative stress; Antioxidants; Glycosylated haemoglobin

### Introduction

In past few decades type 2 diabetes mellitus is recognised as a worldwide health problem with marked increase in cardiovascular mortality and morbidity [1]. Diabetes is also largely responsible for blindness, amputations and end stage renal disease [1]. World Health Organization has predicted a global increase in diabetes prevalence by 39% between the years 2000 and 2030, representing a global increase to 366 million people by the year 2030 [2]. Age, ethnicity, obesity (BMI and waist circumference), blood pressure, fasting blood glucose and lipid concentrations are identified as risk factors for diabetes [3]. Hence it is important to identify and treat diabetes mellitus in early stages, to prevent progression of its various complications.

Hyperglycemia, the primary clinical manifestation of diabetes has been accepted as being essential for development of diabetic complications. Evidence has indicated that some biochemical pathways associated with hyperglycemia like glucose autooxidation, non-enzymatic glycation of proteins and lipids, sorbitol pathway can increase production of free radicals in diabetes mellitus [4]. Free radicals cause oxidative damage to DNA, carbohydrates, proteins and lipids that are normally counteracted by protective antioxidants. A variety of antioxidants exist in body, major of them include superoxide dismutase, glutathione, glutathione peroxidase, catalase, vitamin A, vitamin C and E. Abnormally high levels of free radicals can decline antioxidant levels in the body leading to oxidative stress [5]. Oxidative stress has been implicated in many diseases associated with ageing and in ageing process itself [6]. Oxidative stress has been reported to increase in elderly subjects possibly arising from an uncontrolled production of free radicals by ageing mitochondria and decreased antioxidant defences.

Literature shows that physical inactivity is one of the major risk factors for the development of type 2 diabetes mellitus and

its complications [7]. American Diabetes Association (ADA) and European Association for the study of Diabetes (EASD) recommend weight loss for overweight (BMI  $\geq 25$  kg/m<sup>2</sup>) and obese (BMI  $\geq 30$  kg/m<sup>2</sup>) individuals with diabetes. They specifically note that among these patients relatively small changes in body weight that are achieved through intensive lifestyle interventions can improve fitness, glycemic control and cardiovascular risk factors [8,9]. Lifestyle interventions including exercise have been effective in offsetting type 2 diabetes mellitus complications and progression from prediabetes or metabolic syndrome to type 2 diabetes mellitus [10,11].

Yoga is a mind-body practice incorporating physical postures, breathing exercises and meditation [12]. Yoga may have a different effect on the sympathetic nervous system (SNS) and hypothalamus-pituitary-adrenal (HPA) axis response to stress compared to other types of exercise. Where other exercise can stimulate the SNS/HPA axis, yoga may be down regulating it, shifting to para sympathetic dominance of the stress response [13,14]. Study conducted by Mc Dermott et al. showed that 8 week yoga programme in type 2 diabetes mellitus can significantly reduce body weight, waist circumference, BMI, blood pressure, total cholesterol and perceived stress [15]. Few studies have reported that yoga can be a useful therapy to control oxidative stress

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and blood glucose in type 2 diabetes mellitus [16-19]. In our previous study, we found 3 month yoga intervention to be helpful in reducing BMI, glycemic index and oxidative stress in 60 type 2 diabetes mellitus patients who were on standard care treatment [19]. Since oxidative stress is a part of diabetes process as well as ageing, elderly patients with diabetes mellitus may have higher levels of free radicals compared to younger patients. As this issue was not previously been addressed, in the current study we found it important to analyse the effect of yoga on oxidative stress parameters in two different groups-elderly and younger type 2 diabetes patients.

The present study is aimed to compare the effect of 3 month yoga on oxidative stress and antioxidant parameters in elderly type 2 diabetes patients and younger diabetes comparison group. The secondary objectives were to examine the therapeutic effect of yoga on BMI, blood pressure, fasting plasma glucose and glycosylated haemoglobin levels.

## Methods

The present study was conducted at two yoga centres of Kasturba Medical College Hospital, Mangalore. Patients for this study were recruited from diabetes out-patient department of the hospital. Study was approved by the Institutional Ethical Board. Study population comprised of adults with type 2 diabetes, aged 40-80 years. Cases were the elderly type 2 diabetes patients (n=42) above the age of 60 years and younger comparison group (n=45) were patients with age ≤ 59 years. Other inclusion criteria were: non-alcoholic, non-smoker, stable medication for the last 30 days, willingness to engage in yoga intervention and a working telephone. Exclusion criteria were: acute macron vascular complications, cancer, pulmonary tuberculosis, rheumatoid arthritis, and history of osteoporosis, infection, current participation in any weight loss programme or planning to enrol in any such program during the study participation. Participants enrolled to both the groups were instructed to follow their regular diet for the next 3 months. No recommendation was made by the physician regarding the use of vitamin supplements during the study period. Drug dosages with regard to diabetes and hypertension management were kept stable throughout the study period.

Interested and apparently eligible participants were invited for a baseline evaluation at the study centre. Baseline evaluation included complete medical history, blood glucose measurements, ECG, serum creatinine, fasting lipid profile, micro albuminuria and detailed dilated fundus examination to suggest any complication in diabetes mellitus. At the visit, the researcher described the study design and obtained informed consent.

## Yoga intervention

Participants were allowed to attend yoga classes at any one of the centres managed by the institution according to their convenience. Both young and elderly diabetes subjects attended classes in the yoga centres. Practice sessions consisted of 75-90 min of yoga every day with a weekend break. Yoga intervention consisted of a set of asanas, pranayama and shavasana [20] (Table 1). Floor exercises (vajrasana, pawanamuktasana, bhujangasana, shalabasana, dhanurasana and viparitarani) were waived for patients with heart disease. Patients attending these classes were free to practice these asanas at their homes. Compliance to intervention was defined as attendance for at least 3 days/week at the yoga centre for 3 months as documented by the yoga instructor.

## Outcome measures

Assessments were conducted at baseline and at follow-up (3

SANSKRIT	ENGLISH	TIME DURATION
Tadasana	Mountain pose	2 min
Padahasthasana	Forward bend pose	3 min
Vrikshasana	Tree pose	3 min
Trikonasana	Triangle pose	3 min
Parshvothanasana	Extreme sideways stretch pose	3 min
Vajrasana	Thunderbolt pose	3 min
Vakrasana	Twist pose	4 min
Gomukasana	Cow-faced pose	4 min
Paschimotasana	Seated forward bend pose	4 min
Uttanapadasana	Raised feet pose	4 min
Pawanamuktasana	Wind relieving pose	5 min
Bhujangasana	Cobra pose	3 min
Shalabasana	Locust pose	3 min
Dhanurasana	Bow pose	3 min
Viparitarani	Inverted action pose	4 min
Sitkari pranayama	Teeth hissing	8 min
Bhramari pranayama	Bee breathing	6 min
Anuloma viloma	Alternate nostril breathing	10 min
Shavasana	Corpse pose	10 min

**Table 1:** Set of asanas and pranayama included in the yoga intervention.

months). Physical examination in participants included weight, height and blood pressure measurements. Height was measured in centimetres (cm) and weight in kilograms (kg) using electronic scales. BMI was calculated by weight in kilograms divided by the square of height in meters. Blood pressure was measured in a resting seated position. For the biochemical investigations 7 ml of venous blood was collected in EDTA and fluoride tubes and immediately centrifuged to separate cells and plasma. Plasma was analysed for glucose and vitamin C. From the packed cells, red blood cell hemolysates were prepared to analyse malondialdehyde and reduced glutathione levels.

Malondialdehyde (MDA) a marker of oxidative stress was measured according to Stocks and dormandy method [21]. Under acidic conditions MDA reacts with thiobarbituric acid reagent to give a pink colour which is estimated at 535 nm spectrophotometrically. The method of Beutler et al. was employed to estimate reduced GSH in the blood [22]. This is based upon the development of yellow coloured complex when 5,5'-dithiobis-2-nitrobenzoic acid reacts with sulphhydryl compounds. Vitamin C was estimated by 2,4-dinitro phenyl hydrazine (DNPH) method [23]. In this method plasma ascorbic acid is oxidized by Cu (II) to form dehydroascorbic acid which reacts with acidic 2,4-DNPH to form red bis-hydrazone (520 nm). Plasma glucose and glycosylated haemoglobin (HbA<sub>1c</sub>) were assayed by standard laboratory method in Hitachi 917 random access chemistry analyser.

## Statistical Analysis

Data was analysed by intention-to-treat. All parameters were analysed using SPSS version 16.0. Paired 't' test was used to compare all continuous variables from baseline to follow-up. Mann-Whitney U test, a non-parametric test was used to compare the differences in various parameters before and after intervention between the two groups. Karl Pearson's coefficient of correlation was applied to find the correlation between the variables. A p value <0.05 was considered statistically significant.

## Results

Average attendance at yoga classes was 90-95%. Group 1

comprised of younger diabetes subjects and group 2 were the elderly diabetes individuals. Two participants withdrew from group 1 and three from group 2. The reason for withdrawal was lack of interest in continuing in the study and unexpected travel to another place. A total of 82 patients completed the study. No adverse events were observed during the intervention period. The mean age ( $\pm$  SD) in group 1 was 51.63 ( $\pm$  5.66) years and in group 2 was 67.87 ( $\pm$  5.78) years. The number of males/females recruited for the study was 20/25 in group 1 and 19/23 in group 2. The duration of diabetes was 5.85  $\pm$  4.23 years in group 1 and 10.24  $\pm$  10.35 years in group 2. The duration of hypertension was 1.56  $\pm$  2.49 years in group 1 and 4.30  $\pm$  5.87 years in group 2.

Baseline data showed a positive correlation between age and MDA ( $r$  +0.274) (Table 2). There was a negative correlation between age and antioxidant levels of GSH ( $r$  -0.077) and vitamin C ( $r$  -0.083). Malondialdehyde and antioxidants correlated negatively, i.e., with GSH ( $r$  -0.186) and vitamin C ( $r$  -0.012).

In group 1 there was a significant reduction in mean BMI at the end of 3 months ( $p$ <0.001) when compared to baseline (Table 3). Group 2 also showed a significant reduction in mean BMI at the end of 3 months ( $p$ =0.004). Baseline mean BMI values were comparable between the groups. There was no significant difference between the groups in the amount of BMI reduction experienced by each group post-intervention. Both the groups showed a significant reduction in mean blood pressure and there was no inter-group difference at the end of the study. Fasting plasma glucose values improved in both group 1 and 2. There was no significant difference between the groups. The mean concentration of HbA<sub>1c</sub> significantly differed between group 1 and group 2 ( $p$ <0.05) and in latter group it increased marginally from 8.5  $\pm$  1.3% at baseline to 8.6  $\pm$  1.7% after 3 months. Oxidative stress as indicated by MDA significantly decreased in both the groups, with reductions of 18% and 22% in group 1 and group 2 ( $p$ <0.001). Glutathione level significantly improved by 15% in group 1 ( $p$ =0.005). Group 2 also showed a significant rise in GSH level by 10% at follow-up ( $p$ =0.021). However there was no significant difference between the two groups. Vitamin C showed a significant improvement in group 1 ( $p$ <0.05) when compared to group 2.

Variables	Age	Glutathione	Vitamin C
Malondialdehyde	0.274	-0.186	-0.012
Age		-0.077	-0.083

**Table 2:** Pearson correlation coefficients for variables- (i) Malondialdehyde with age, antioxidants (ii) Age with antioxidants.

Variables	Group 1 (n=45)			Group2 (n=42)			p value
	Baseline	After 3 months	p value	Baseline	After 3 months	p value	
BMI (kg/m <sup>2</sup> )	26.1 $\pm$ 3.7	25.0 $\pm$ 3.6	<0.001	26.0 $\pm$ 3.3	25.5 $\pm$ 3.2	0.004	0.972
BP systolic (mm Hg)	134 $\pm$ 18.2	130.7 $\pm$ 13.9	<0.05	141.4 $\pm$ 17.0	135.8 $\pm$ 13.4	<0.001	0.123
BP diastolic (mm Hg)	82.15 $\pm$ 8.5	79.2 $\pm$ 8.9	<0.05	83.4 $\pm$ 10.1	80.4 $\pm$ 5.6	<0.05	0.335
Fasting glucose (mmol/L)	7.5 $\pm$ 2.5	6.8 $\pm$ 2.2	0.017	8.6 $\pm$ 2.7	7.7 $\pm$ 2.3	0.006	0.119
HbA <sub>1c</sub> (%)	8.5 $\pm$ 1.4	7.2 $\pm$ 1.2	<0.05	8.5 $\pm$ 1.3	8.6 $\pm$ 1.7	0.644	<0.05
Malondialdehyde ( $\mu$ mol/L)	56.0 $\pm$ 9.6	46.0 $\pm$ 9.8	<0.001	50.0 $\pm$ 12.0	39.0 $\pm$ 9.0	<0.001	0.079
Glutathione ( $\mu$ mol/gm Hb)	7.5 $\pm$ 2.4	8.6 $\pm$ 2.5	0.005	7.4 $\pm$ 2.9	8.1 $\pm$ 2.5	0.021	0.678
Vitamin C ( $\mu$ mol/L)	23.2 $\pm$ 10.2	31.8 $\pm$ 18.2	0.045	34.6 $\pm$ 36.9	41.5 $\pm$ 22.7	0.428	0.079

**Table 3:** Comparison of selected clinical and oxidative stress variables among group 1 and group 2 type 2 diabetes subjects engaged in yoga intervention. Group 1: Young type 2 diabetes; Group 2: Elderly type 2 diabetes Data are mean  $\pm$  SD p values are significance values

## Discussion

The results of the present study are encouraging and reveal that yoga might be an effective adjunctive treatment for reduction of oxidative stress in type 2 diabetes patients. In this study we observed a positive correlation between age and oxidative stress in diabetes patients. The results are in accordance with earlier report showing that oxidative stress increase with age [24]. Oxidative stress also aggravate with the complications of the disease [1,25]. Hence elderly diabetes subjects are at greater risk for oxidative stress or diabetes related complications which might be a result of high oxidative stress in them.

Previous studies on yoga reveal that yoga is an effective intervention to control oxidative stress in type 2 diabetes mellitus [18,19,25,26]. In our previous study we found a significant reduction in oxidative stress and improvement in many antioxidant parameters including glutathione and vitamin C [19]. The study participants were both young and elderly type 2 diabetes patients enrolled in yoga intervention. Because of the smaller sample size we were not able to find effect of 3 month yoga practice on sub-groups of young compared to elderly diabetes patients with complications. When compared to our earlier study on yoga, in the present study we found a significant improvement in oxidative stress in both groups irrespective of the age. There was also a rise in glutathione level in these groups. Younger diabetes subjects were able to achieve a substantial increase in vitamin C level, whereas there was no improvement in vitamin C status of elderly diabetes subjects.

The present study also showed that 3 month yoga can reduce BMI and blood pressure in diabetes subjects irrespective of the age. Yoga was found to be beneficial in improving fasting glucose values in these subjects. However younger diabetes patients were able to achieve a significant reduction in HbA<sub>1c</sub> levels when compared to elderly subjects. In elderly group there was no improvement in glycemic status. This finding could be due to the reason that many of the elderly subjects with macrovascular disease were restricted from performing floor yoga asanas. It was also observed that stretching and bending of the body during these asanas was limited in these elderly subjects. It could be possible that these movements of yoga may exert a massaging effect on various internal organs, inducing release of insulin from pancreas.

## Possible mechanisms of action

It seems likely that yoga improves oxidative stress and other indices of diabetes management by two important mechanisms. First by reducing over activation of hypothalamo-pituitary-adrenal axis and second by stimulation of parasympathetic nervous system by slow rhythmic breathing in yoga [27,28].

## Limitations

The major limitation of the study was that the investigator analysing oxidative stress and antioxidant parameters was not blinded. Due to lack of resources and staff for this study, it was not feasible to conduct a single-blind study. Other laboratory estimations like fasting glucose and HbA<sub>1c</sub> was carried out at national accredited laboratory of the hospital. The personnel's doing the test was blinded to the study protocol. Since benefit of yoga is widely recognized among people and participants were aware that what they were practicing was yoga, this might have created an outcome expectation among them. The improvement in oxidative stress index, i.e., MDA could have been partly due to this. Involvement in an active intervention like yoga might indirectly encourage participants to lead a healthy lifestyle with good eating pattern. This factor might also compromise the validity of our findings. Thus it was important to analyse the diet pattern in these participants both at pre and post-test. Another limitation of this study was that participants had multiple diagnoses that may have complicated the interpretations of the research outcomes.

## Implications and scope for future study

Our study has several implications for future investigations of yoga based interventions in type 2 diabetes mellitus. Elderly type 2 diabetes patients may have an increased risk for oxidative stress mediated diabetes complications in them. The present study results reveal that 3 month yoga practice may be a beneficial treatment for diabetes patients to reduce oxidative stress. The findings of this study need to be confirmed in larger and more sufficiently powered studies. In future studies, it would be important to evaluate how long the benefits lasted for the participants and which asanas are most beneficial for diabetes patients.

## Conclusion

In summary, 3 month yoga is a feasible intervention and may help reduce oxidative stress, blood pressure and BMI in diabetes patients. Elderly diabetes subjects have higher oxidative stress with diminished antioxidant status when compared to their younger counterparts. Younger diabetes subjects achieved a significant improvement in vitamin C and glycemic status after 3 month yoga. The data supports promise for yoga-based intervention in type 2 diabetes, and suggest that further optimization of intervention content and duration may be useful.

## Conflict of Interest Statement

No potential conflicts of interest relevant to this article were reported.

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