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Improvement of Glutathione and Total Antioxidant Status with Yoga

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ABSTRACT

Objective: Several studies suggest that yoga can decrease oxidative stress. However reports are scanty regarding whether yoga training can improve the glutathione level of individual. This study is designed to appraise the role of yoga in maintaining glutathione (reduced and oxidized) levels and antioxidant status.

Study design: This study was conducted on healthy male volunteers from the Indian Navy, who were divided into two groups—a yoga (n = 30) group and a control (n = 21) group. The yoga group was trained in yoga for 6 months. The yoga schedule consisted of prayers, *asana*, *pranayama*, and meditation. The control group practiced routine physical training exercise for 6 months. Blood samples were collected when the volunteers were in fasting condition before and after completion of 6-month training period. Reduced and oxidized glutathione, glutathione reductase activity and total antioxidant status (TAS) were estimated.

Results: Reduced glutathione level increased significantly ($p < 0.05$) in the yoga group after completion of training. Glutathione reductase activity increased significantly in the control group ($p < 0.05$). TAS increased significantly ($p < 0.001$) in the yoga group and decreased significantly ($p < 0.001$) in the control group.

Conclusions: Regular practice of yoga can maintain or improve antioxidant level of the body. The clinical relevance is that yoga practice can be used to maintain the antioxidant defense system under stressful conditions of training as observed in the case of soldiers and athletes.

INTRODUCTION

Yoga is known for its beneficial effects on physiologic and psychologic functions.[1–8] During the last 3 decades, extensive physiologic research have been done on yogic practices. It has been reported that yoga can increase muscular efficiency, endurance time[9] and aerobic capacity, and can reduce perceived exertion after exercise.[10–12] Yoga is widely used as a stress reliever.[13–18] Additionally, yoga has a profound effect on the autonomic nervous system (ANS)[3,4,19–23] and may reduce stress levels in individuals via this effect. Regarding oxidative stress, reports have suggested that yoga can decrease oxidative stress,[24,25] reduce the malonaldehyde (MDA) level, and increase superoxide dismutase (SOD) and catalase activity.[1] Various reports regarding aerobic exercise training propose that mainly catalase activity is increased by training[26] but exercise training is not sufficient to maintain the redox status of the body. There is growing evidence that supports the beneficial effect of yoga on antioxidant enzymes. But reports regarding the role of yoga on glutathione (a major antioxidant tripeptide) level in reducing oxidative stress are scanty. Therefore the present study was undertaken to evaluate the effect of yoga on glutathione level and total antioxidant status (TAS).

MATERIALS AND METHODS

Subjects

The study was conducted on healthy male volunteers of the Indian Navy belonging to various branches (i.e., ship,

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submarine, aviator, etc.). Initially, 60 volunteers were randomly selected from the personnel of the Indian Navy. The volunteers were briefed about the study protocol approved by the ethical committee of the Defence Institute of Physiology and Allied Sciences, Delhi, India, and written consent was obtained from each volunteer. Then the volunteers were divided into two groups: a yoga group and a control group. The yoga group consisted of 30 volunteers (age: 32.8 $\pm$ 1.4 yrs; height: 168.8 $\pm$ .9 cm; weight: 65.2 $\pm$ 1.5 kg) and the control group consisted of 21 volunteers (age: 25.5 $\pm$ 1.6 yrs; height: 170.1 $\pm$ 0.9 cm; weight: 62.7 $\pm$ 1.8 kg). Initially, there were 30 volunteers in each group, but 9 volunteers dropped from the control group and could not continue the entire 6 months of training. Thus, the control group completed the study with 21 volunteers. As the volunteers had free choice to select which group to be in, the majority of the older persons among the volunteers preferred to do yoga, as this is a comparatively lower-intensity form of exercise. Thus, the mean age of the yoga group was comparatively higher ($p < 0.05$) than that of the control group. For this reason, each group was compared individually at baseline and after 6 months.

Yoga instructors

Twenty Naval personnel of the Eastern Naval Command were chosen for training as yoga instructors. The professional instructors of the Kaivalyadhama Institute, Lonavala, Maharastra, India, trained the personnel in yoga for 2 months. The training schedule included practical *asanas* and theory lectures. The trainers taught both theory and practical aspects of yoga and its benefits to the volunteers (Table 1).

Study design

The yoga group underwent yogic training, for 1 hour in the morning, 5 days per week for 6 months. The yoga schedule consisted of prayers, *asanas*, *pranayama*, and meditation. Each *asana* was performed for 1–2 minutes' duration (Table 2). At the end of the *asana*, various breathing maneuvers (*pranayama*) were performed for 5–10 minutes. *Pranayama* included deep breathing, inhalation-retention-exhalation with a ratio of 1:1:2, abdominal (diaphagramatic) breathing, and alternate nostril breathing. Breathing exercises were recommended immediately after practicing *asanas*. This was then followed by meditation for 5 minutes. Meditation was a component of Patanjali's *Astanga* yoga. During meditation the individuals sat in a comfortable posture (either *Sukhasana* or *padmasana*) with eyes closed and tried to feel completely relaxed. First, the subjects followed the usual wandering mind/thought process that allows the mind freedom. Later, slowly, they tried to be aware of their surroundings. Subsequently, the subjects tried to pay attention, slowly, to different body parts from toe to head and, ultimately, to the breathing process (i.e., awareness of a normal breathing cycle). After this the subjects chanted the *Omkar* Mantra (the syllable *AUM*) and tried to feel the presence of the Almighty. The pattern was, to some extent, similar to a meditation technique mentioned in a paper by Wallace and Benson.[7] The control group practiced routine physical training (PT) exercises during the same period. The PT schedule (total 1 hour) included slow running up to 4 km (30 minutes), body-flexibility exercises (10 minutes), pull ups (5 minutes), and games (15 minutes). On completion of 1 hour of daily training, both groups returned to their

TABLE 1. PROTOCOL AND TRAINING SCHEDULE FOR YOGA INSTRUCTORS

Practical asanas—2 hrs *Theory lectures—3 hrs* *Practical asanas—1 hrs*	
1 month	*2 months*
Ardha Halasana with one leg 3 times	*Halasana*
Pavan Muktasana with one leg 3 times	*Pavan Muktasana*
Bhujanganasana	Crocodile poses
Shalabhasana with one leg	*Paschimtanasan*
Parvatasan	*Suptavajrasan*
Vajrasan	*Sarvanrasana*
Tadasan	*Matsyasana*
Chakrasan	*Dhanurasan*
Pada hastasan	*Setubandasan*
Trikonasan	*Gomukasan*
Brahma Mudra	*Vrikshasan*
Yoga Mudra	*Santulasan*
Anuloma Viloma	*Utkatasan*
Kapalbhati	*Agnisar Kriya*
	Bandhas—Moll band *uddiya, Jalandar*
	Bhastrika
	Ujjayi
	Suryabhedan

TABLE 2. PROTOCOL AND SCHEDULE FOR YOGA TRAINING

Duration: 1 hour	
Time: 6:30–7:30 AM	
1st month	*2nd–6th months*
Ardha Halasana	*Kapalbhati*—2 minutes
Pavan Muktasana	*Surya Namaskar*—2 rounds
Bhujanganasana	*Asanas* (30 minutes)
Shalabhasana	*Padmasana*
Parvatasana	Yoga *Mudra*
Vajrasana	*Paschimottanasana*
Tadasana	*Pavanamuktasana*
Chakrasana	*Dhanurasana*
Padahastasana	*Suptavajrasana*
Trikonasana	*Gomukasana*
Brahmamudra	*Halasana*
Yoga *Mudra*	*Karmapadasana*
Anuloma-viloma	*Bhujangasana*
Kapalbhati	*Uttanpadasana*
	Pranayama (15 minutes)
	Bhastrika—3 minutes
	Anulom-viloma—5 minutes
	Kakimudra—2 minutes
	Bhramari—5 minutes
	Meditation—2 minutes

normal daily routines in their respective units. This routine PT schedule was followed by both of the groups before the commencement of the 6 month training schedule.

Collection of samples

Fasting blood samples were collected when the volunteers were in resting condition in the morning hours (at 7:00–8:00 AM) before and after completion of 6 months of training and analyzed for reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase (GR), activity, and TAS.

Measurement procedures

For the estimation of GSH and GSSG, blood samples were collected in 10% (w/v) metaphosphoric acid and estimated using the fluorimetric method of Hissin and Hilf.[27] This method measures both GSH and GSSG with an *o*-phthalaldehyde (OPT) as a fluorescent reagent. Briefly, metaphosphoric acid-treated whole blood was centrifuged and the supernatant was treated with OPT at a pH of 8 and estimated for GSH using a fluorescence spectrophotometer with an excitation and emission wave lengths of 350 nm and 420 nm. The same process was followed for GSSG at pH 12. For estimating GSSG, GSH can be complexed to *N*-ethylmaleimide to prevent interference of GSH with the measurement of GSSG.

To measure GR activity, whole blood was taken in an EDTA (ethylenediaminetetraacetic-acid)-treated vial and a 10% lysate of whole blood was prepared to measure the activity spectrophotometrically by the method of Racker.[28] Via this method, lysate was incubated with GSSG and NADPH (nicotinamide adenine dinucleotide phosphate reduced) at a pH 7.5 and changes in optical density were measured at 340 nm for 3 minutes at an interval of 30 seconds. The results were expressed as μmol of NADPH oxidized per minute per mL of lysate.

TAS was measured as an ABTS (2,2′-azino-di[3-ethylbenzthiazoline sulphonate) radical cation decolorizing assay using a Randox kit (Cat No. NX 2332) (Randox Laboratories Ltd., Ardmore, U.K.).

Statistical analysis of the work was carried out by a Student's paired *t* test.

RESULTS

In the yoga group, GSH level increased significantly ($p < 0.05$) from the baseline value of 235.3 $\pm$ 16.9 nmol/L to 331.7 $\pm$ 37.6 nmol/L after completion of training. In the control group, the GSH level decreased. Values of GSSG decreased in the yoga group and increased in the control group. The ratio of GSH/GSSG increased significantly ($p < 0.001$) from the pretraining value of 0.88 $\pm$ 0.02 to 1.34 $\pm$ 0.04 in the yoga group. In the control group, this ratio did not decrease significantly. GR activity increased significantly ($p < 0.05$) from baseline value of 0.82 $\pm$ .05 μmol/mL/min to 0.98 $\pm$ 0.06 μmol/mL/min after completion of training in the control group. TAS significantly ($p < 0.001$) increased from the pretraining value of 1.23 $\pm$ .04 mmol/L to the post-training value of 1.96 $\pm$ 0.03 mmol/L in the yoga group and decreased significantly ($p < 0.001$) from the pretraining value of 1.37 $\pm$ 0.05 nmol/L to the post-training value of 1.06 $\pm$ 0.04 nmol/L in the control group. Comparisons of various parameters between the two groups are presented in Table 3.

DISCUSSION

GSH is a very important substrate for antioxidant defense system and is used by glutathione peroxidase as a donor of a hydrogen atom to reduce hydrogen peroxide (H_2O_2) into H_2O.[29] An increased amount of GSH is very helpful for an antioxidant defense mechanism that reduces oxidative stress.[30] GSH is oxidized to GSSG in cells in response to an increase in free radicals in a state of oxidative stress.[31] It is well-recognized in the literature that acute bouts of exercise can change the glutathione redox status of the cells toward an oxidized state (i.e., a decrease in GSH level and an increase in GSSG level).[32–35] In the case of training, reports suggest that aerobic training can increase the production of oxygen-free radicals and that oxidative stress is likely because of electron leakage at intermediary steps in the elec-

Table 3. Comparison of Various Oxidative Stress–Related Parameters in Yoga and Control Group at Baseline and After Training

Yoga group			
Parameters	*Baseline*	*After 6 months*	p-*value*
GSH (nmol/L)	235.3 ± 16.9	331.65 ± 37.6	$p < 0.05$
GSSG (nmol/L)	263.2 ± 9.4	255.8 ± 10.8	Not significant
GSH/GSSG	0.88 ± 0.02	1.34 ± 0.004	$p < 0.001$
GR activity (mmol/mL/min)	0.89 ± 0.05	0.88 ± 0.05	Not significant
TAS (mmol/L)	1.23 ± 0.04	1.96 ± 0.03	$p < 0.001$
Control group			
Parameters	*Baseline*	*After 6 months*	p-*value*
GSH (nmol/L)	328.1 ± 28.5	288.6 ± 18.7	Not significant
GSSG (nmol/L)	294.3 ± 9.3	314.6 ± 18.5	Not significant
GSH/GSSG	1.19 ± 0.07	0.98 ± 0.12	Not significant
GR activity (mmol/mL/min)	0.82 ± 0.05	0.98 ± 0.06	$p < 0.05$
TAS (mmol/L)	1.37 ± 0.05	1.06 ± 0.04	$p < 0.001$

Value: Mean ± standard error of the mean.
GSH, reduced glutathathoine; GSSG, oxidized glutathione; GR, gluthathione reductase; TAS, total antioxidant status.

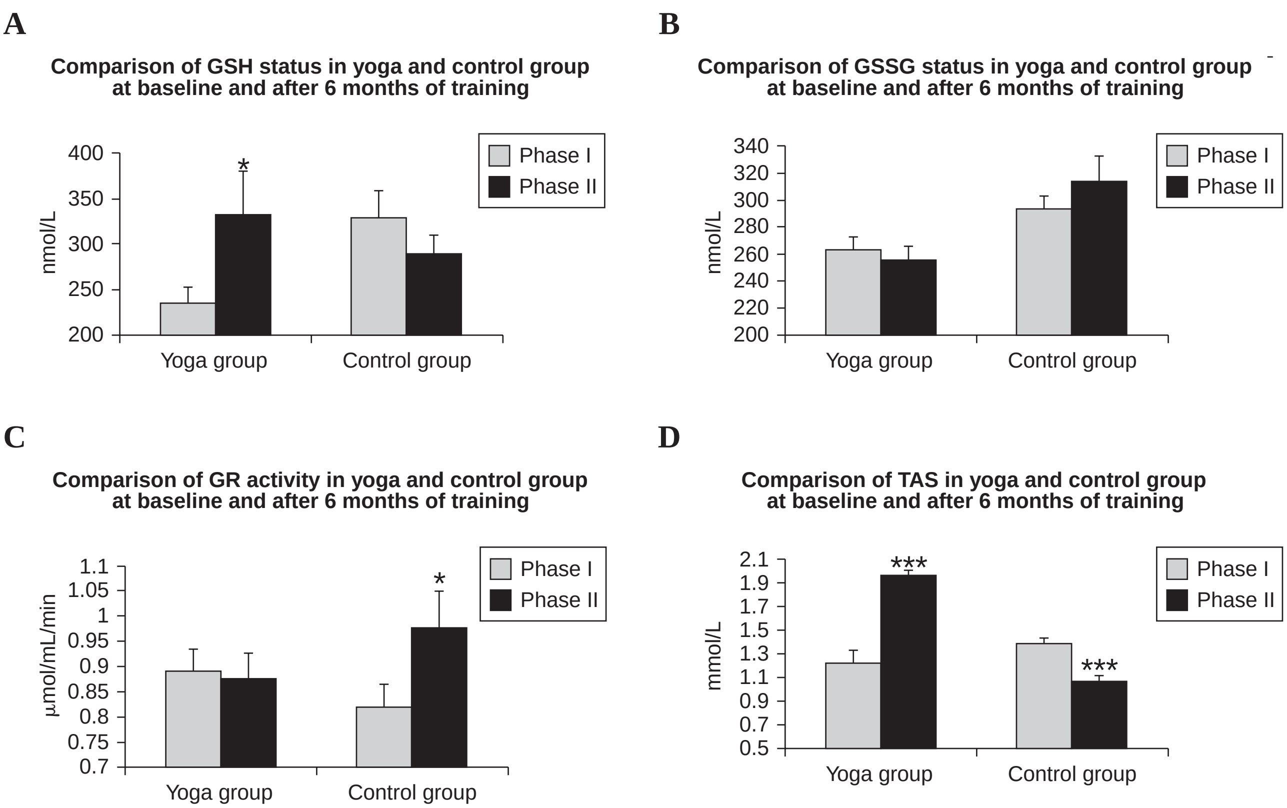

FIG. 1. Comparison of the levels of reduced glutathione **(A)**, oxidized glutathione **(B)**, and glutathione reductase **(C)**, as well as total antioxidant status **(D)** in the yoga and control groups. Phase I is before training. Phase II is after 6 months of training. $*p < 0.05$; $***p < 0.001$.

tron-transport chain. Over the past decade, numerous studies examined exercise training as an oxidative stressor to evaluate the ability of the body to respond to the potential oxidative stress of training via positive adaptation to its antioxidant defense. However, there was a lack of antioxidant adaptation to aerobic and anaerobic training at substrate level in humans.[26,36] Similarly, our results did not show this kind of positive adaptation in the control group after 6 months of training. Proper positive adaptation might depend on the intensity and duration of training used. These results suggest that the aerobic training to which volunteers were exposed might not be sufficient enough to induce positive adaptation.

In this study, we have seen that GSH level increased significantly in the yoga group ($p < 0.05$) and decreased in the control group. However, GSSG levels decreased in the yoga group and increased in the control group but these changes were not significant. This suggests that redox status can be shifted toward a reduced state by practicing yoga, which is an important adaptative strategy for minimizing oxidative stress and its effects. GSH/GSSG ratio is another important and sensitive marker of the antioxidant system. This ratio increased significantly in the yoga group, which confirms further the beneficial effect of yoga training on the antioxidant system. A decreased GSH/GSSG ratio in the control group indicated a decrease in the reductive capacity of the red blood cells.

Generally, aging persons are more susceptible to oxidative stress. In this study, in spite of the subjects of the yoga group being comparatively aged (32.8 ± 1.4 yrs) than the control group (25.5 ± 1.6 yrs), the yoga group had a better capacity to combat oxidative stress. This reaffirms that yogic practices help in the management of oxidative stress.

Among antioxidant enzymes, GR is a primary enzyme for maintaining glutathione redox status. It converts GSSG to its reduced state (GSH). In the process of reduction GR uses NADPH as a hydrogen donor.[29] Previous reports proposed enhancement in GR activity after exercise.[37,38] Our study also showed the same trend. Here, in the control group GR activity increased significantly as a result of increased availability of its substrate (i.e., GSSG). But this increased GR activity was not sufficient enough to maintain the body milieu in a reduced state, as a result GSSG levels were still high in the control group. However, decreased levels of GSSG and GR activity in the yoga group again showed a positive response to yoga on the part of the antioxidant system.

TAS of a cell represents overall antioxidant capacity of the cell. It has been suggested that oxidative stress occurs during physical exercise.[39,40] In this study, significant increase in TAS in the yoga group clearly showed a marked improvement of overall cellular antioxidant level. However, significant reductions in TAS in the control group showed diminished antioxidant capacity, an effect that makes an individual affected more by the deleterious effects of oxidation.

To achieve better comprehension we did intergroup comparisons and these also showed a supportive result for the yoga group. In the GSH value at baseline, there was a marked difference ($p < 0.001$) between the two groups and the redox status was much better in the control group, which had higher GSH levels. This could be attributed to the young age of the subjects in that group. But this trend reversed after 6 months of yoga training. Yoga augmented the GSH levels significantly ($p < 0.05$) in the yoga group compared to the control group. For GSSG, there was no such significant difference at baseline between the two groups. But after training, GSSG levels increased significantly ($p < 0.001$) in the control group in contrast to the yoga group. GR activity also showed a similar trend. TAS value was moderately higher in the control group almost approaching significance ($p = 0.07$) at baseline. But after yoga training, an enormous raise in TAS value was seen in the yoga group ($p < 0.001$) compared to the control group. All these results are clearly evidence of the fact that, despite being older, volunteers in the yoga group had better antioxidant adaptation after performing 6 months of training.

CONCLUSIONS

According to the findings of this study, it may be concluded that yoga may upregulate the antioxidant capacity of cells to combat oxidative stress.

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