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Biological Psychology 63 (2003) 281–291
BIOLOGICAL PSYCHOLOGY
www.elsevier.com/locate/biopsycho

Sudarshan Kriya practitioners exhibit better antioxidant status and lower blood lactate levels

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Received 22 September 2002; accepted 28 February 2003

Abstract

Oxidative stress may contribute to the pathophysiology of many chronic diseases. Since psychosocial stress increases oxidative stress, we conducted an exploratory study to investigate the effects of stress reduction with the Sudarshan Kriya (SK) program, on superoxide dismutase (SOD), catalase, glutathione and blood lactate levels in practitioners and non-practitioners of SK. Blood samples of ten practitioners of SK and 14 non-practitioners of any formal stress management technique were analyzed for SOD, catalase, glutathione and lactate levels. Differences between groups and subgroups were analyzed by t-test and correlations between variables compared using Pearson's correlation coefficient. Significantly lower levels of blood lactate ($P = 3.118e-10$) and higher levels of SOD ($P = 0.0001415$), glutathione ($P = 2.038e-06$) and catalase ($P = 0.001565$) were found in practitioners as compared to non-practitioners of SK, thereby suggesting that lower levels of blood lactate and better antioxidant status in practitioners are associated with regular practice of SK technique. However, this study needs to be conducted on a larger sample size to confirm this effect.

Keywords: Catalase; Glutathione; Lactate; Sudarshan Kriya; Superoxide dismutase

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0301-0511/03/$ - see front matter
doi:10.1016/S0301-0511(03)00071-1

1. Introduction

Oxygen is both essential and harmful to life. During biological oxidation 4–5% percent of oxygen consumed in respiration is not reduced to water but instead forms free radicals (Halliwell, 1991). Though oxygen is the most critical nutrient of life, it is also the source of reactive oxygen species (ROS), such as hydroxyl ions, superoxide anions and hydrogen peroxide. Oxidative stress is an internal damage caused by ROS. The human body is constantly under the attack of ROS either generated during the normal metabolic process in the cells or by external agents such as radiation, ultraviolet light, cigarette smoke, environmental pollutants like asbestos, pesticides, etc. (Halliwell, 1991; Raij et al., 2001).

An antioxidant is a substance that scavenges ROS. The body is equipped with antioxidant defence in the form of glutathione and antioxidant enzymes i.e. superoxide dismutase (SOD), catalase, glutathione peroxidase. The problem arises when the level of ROS increases so much that the body antioxidant defence mechanism is not able to counteract the ROS. ROS/ free radicals can damage cellular functions and cause peroxidation of lipids in the cellular membrane (Murin et al., 2001; Fujita, 2002; Logani and Davies, 1980). Free radicals are chemical species that possess one or more unpaired electrons, are thus highly unstable and to attain stability, try to gain electrons from other adjoining molecules, and in turn convert them to unstable free radicals. This starts a cascade/chain reaction, leading to more production of free radicals causing more damage to lipids, proteins and DNA in the cells.

There is increasing body of evidence (Scarpellini et al., 1994; Adachi et al., 1993) that chronic psychosocial stress may increase oxidative stress. The hypotheses that free radical mediated oxidation (i.e. oxidative stress) may contribute to the pathophysiology of atherosclerosis, coronary heart disease (CHD), other chronic diseases (e.g. cancer and rheumatoid arthritis) (Sorescu et al., 2002; Penckofer et al., 2002; Cavalca et al., 2001) and the aging process have gained increased acceptance (Wickens, 2001). Thus, psychosocial stress may contribute to the etiology of CHD, other chronic diseases and aging through free radical mechanisms (Schneider et al., 1998; Bland, 1998).

Previous studies report decline in blood lactate during transcendental meditation (TM) possibly due to increased cardiac output and redistribution of circulation resulting in increased muscle perfusion (Jevning et al., 1992, 1978). Other postulated mechanisms suggest decrease in blood lactate due to changes in erythrocyte metabolism. Rapid decrease of whole blood and red cell glycolytic rate has been reported during TM, with slight increase accompanying rest. Since the red cell contributes a major proportion of total blood lactate content in men, it was concluded in these reports that decreased red cell glycolysis probably accounts for TM induced decreased blood lactate (Jevning et al., 1983, 1985).

Sudarshan Kriya (SK) is a breathing technique introduced by Sri Sri Ravi Shankarji and involves breathing in three different rhythms. It is preceded by Ujjayi Pranayam (long and deep breaths with constriction at the base of throat) and

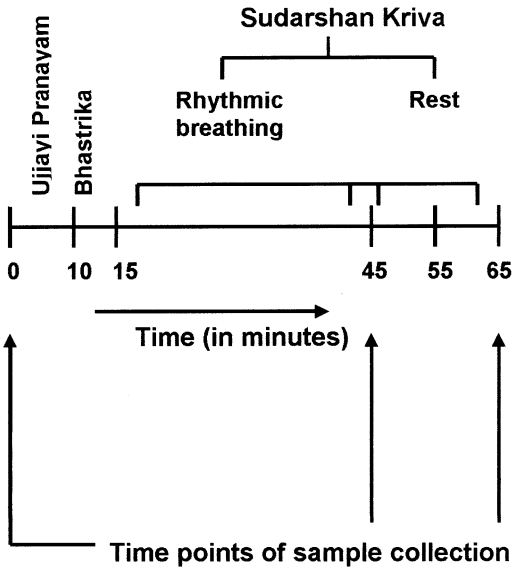

Fig. 1. Diagram showing different stages of SK and various time points of sample collection before and during SK.

Bhastrika (fast and forceful breaths through nose along with arm movements) (Fig. 1) (Janakiramaiah et al., 2000).

There are reports to suggest that stress reduction with the TM program reduces psychosocial stress (Alexander et al., 1994). However, to date, the effects of stress reduction with such stress management techniques on oxidative stress has not been extensively investigated. Therefore, we conducted an initial exploratory study to evaluate antioxidant levels and blood lactate in practitioners of SK as compared to non-practitioners of SK.

2. Methods

2.1. Study group

The 24 subjects were normal healthy males from Police Training College (PTC), Delhi, India. PTC recruits were chosen for this study because they were residing in similar living conditions and also because of the similarity in their anthropometric measurements, demographic and life style characteristics and ate a similar diet. Twenty-four individuals from this college were randomly taken for the study after obtaining the voluntary consent. Out of these 24, 10 were taken for the experimental group (Age range: 22–27 years) and 14 as controls (Age range: 21–27 years). The difference in the two groups was that the experimental group was made to practice SK actively while the control group did not practice SK at all. Their baseline levels of

glutathione, SOD, catalase and blood lactate were estimated before starting the practice and were found to be comparable. The experimental group kept on practicing SK for a period of five months before the samples for the following study were collected. Laboratory conditions (Ambient room temperature with no background noise) for experimental group (while practicing SK) and controls (while relaxing) were the same. Both the groups were tested at the same time of the day and within the same time frame.

2.2. Sample collection

An 18G indwelling three-way cannula was introduced into the median cubital vein of the forearm of practitioners and non-practitioners of SK and kept patent by heparinised saline. Samples were taken immediately before the start of SK practice, after 45 min and after 65 min of starting SK. Three ml of blood was collected in a single attempt at each time point out of which 0.5 ml was transferred to a tube containing 1 ml of 5% perchloric acid. The supernatant was used for lactate estimation. Remaining 2.5 ml was collected in EDTA containing tubes and used for different enzyme assays. The plasma was used for catalase estimation whereas the RBCs were used for SOD and glutathione estimation.

2.2.1. SOD estimation

SOD was estimated using a modified method of Marklund and Marklund (1974). This method utilizes the inhibition of autoxidation of pyrogallol by SOD enzyme. 2.8 ml of Tris buffer containing 50 μM Tris & 1mM EDTA was added to 100 μl of the sample to which 100 μl of pyrogallol was then added. For the control sample 2.9 ml of Tris buffer was added to 100 μl of pyrogallol. The Optical Density (O.D.) was then taken at 420 nm every 30 s for 5 min. The concentration of pyrogallol was adjusted to get the difference in the absorbance (0.020–0.030 nm) at the interval of one minute. The SOD activity was calculated as follows:

$$\text{SOD (U/ml)} = \frac{\delta\text{O.D.}_{\cdot\text{control}} - \delta\text{O.D.}_{\cdot\text{sample}} \times 100 \times 10}{\delta\text{O.D.}_{\cdot\text{control}} \times 50}$$

Each experiment was done in duplicate.

2.2.2. Glutathione estimation

Glutathione was estimated using the method of Tietze (1969). 100 μl of 25% Trichloroacetic acid (TCA) was added to 0.4 ml of plasma. The solution was then centrifuged at 5000 rpm at 4 °C for 15 min. 100 μl of protein free solution was then taken from the supernatant to which 2 ml of 0.6 mM dithionitrobenzidine (DTNB) solution in 0.2 M phosphate buffer, pH 8.0 was added. The volume was made to 3 ml with 0.2 M phosphate buffer. Blank containing 100 μl of 5% TCA and glutathione standard in the range of nanograms were run simultaneously. The optical density (O.D.) was taken at 412 nm. The amount of glutathione was then calculated from the standard curve, and the results expressed as nmoles of glutathione per mg protein.

Protein estimation was done using the Bradford's method (Bradford, 1976). Each experiment was done in duplicate.

2.2.3. Assay of catalase activity

Plasma catalase was estimated using the method of Brannan et al. (1981). Briefly, 50 μl of imidazole buffer (17.14 mM imidazole plus 1% Triton × 100, 0.7% BSA) containing 12 nM H_2O_2 was added to 10 μl of plasma and the volume was made to 100 μl with distilled water. Sample containing 10 μl of 10mM sodium azide and blank containing plasma with only imidazole buffer but no H_2O_2 was also taken. The reaction mixtures were incubated at 25 °C for 25 min. The reaction was stopped by adding 750 μl of stop buffer containing 0.1 M potassium phosphate buffer, pH 7.4, peroxidase (800 U/100 ml), 6 nM 0-dianisidine dimethoxybenzidine and 100% ethanol. The catalase activity was calculated as follows:

$$\text{Activity (units/mg protein/min)} = \frac{\text{o.d.}_{\text{Azide}} - \text{o.d.}_{\text{Blank}}}{\text{o.d.}_{\text{Sample}} - \text{o.d.}_{\text{Blank}}} \times \frac{1}{\text{mg protein}} \times \frac{1}{25\text{min}}$$

Each experiment was done in duplicate.

2.2.4. Lactate estimation

Lactate was estimated using the method of Pesce et al. (1975). 1 ml of perchloric acid was added to 0.5 ml of blood immediately after collection. It was then centrifuged at 4000 rpm for 15 min. 200 μl of supernatant was added to 2 ml glycine buffer containing 20 μl lactate dehydrogenase (LDH). Then 200 μl of nicotinamide adenine dinucleotide (NAD) was be added to the solution at 4 °C. The reaction mixture was then incubated for 1 h at 25 °C. The O.D. was taken at 340 nm against reagent blank containing only perchloric acid. Each experiment was done in triplicate.

2.2.5. Statistical analysis

Mean difference between the various groups was assessed by unpaired and paired '*t*' test. Difference between parameters at various time points of the kriya was tested using ANOVA. Correlation between different parameters was calculated using Pearson's correlation coefficient. A *P*-value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Long term effects of SK on blood lactate, glutathione, catalase and SOD

The basal levels of glutathione were found to be significantly higher ($t = 6.968$, $P = 2.038\text{e}{-06}$) in practitioners (285.3 ± 35.99 nmoles/mg protein) of SK as compared to non-practitioners (68.2 ± 6.7 nmoles/mg protein) (Fig. 2). Similar findings were obtained for the antioxidant enzymes i.e. catalase and SOD. The basal

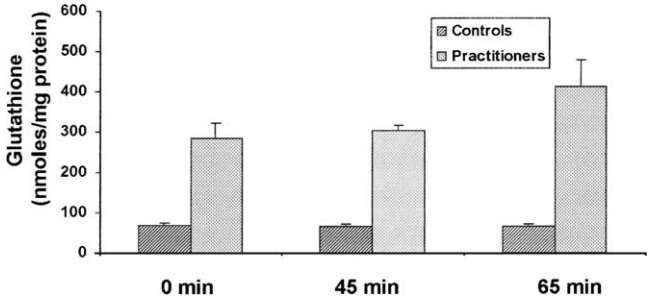

Fig. 2. Bar diagram showing the comparison of glutathione levels (mean ± S.E.) in controls and in practitioners of SK at various time points during the Kriya. The controls did not show a significant change. Note the higher basal level of Glutathione in long term practitioners of SK. Each experiment was done in duplicate.

level of catalase was found to be 1.729 ± 0.178 units/mg protein/min in practitioners whereas it was lower ($t = 3.651$, $P = 0.0016$) in non-practitioners (1.14 ± 0.05 units/mg protein/min) (Fig. 3). Significantly ($t = 4.727$, $P = .0001415$) higher levels of SOD were obtained for practitioners (12.66 ± 1.35 units/ml) as compared to non-practitioners (4.8 ± 1.02 units/ml) (Fig. 4).

Four fold lower levels ($t = -2.265$, $P = 3.118e-10$) of blood lactate were obtained in case of practitioners of SK (0.47 ± 0.032 mmoles/l) as compared to non-practitioners (1.64 ± 0.037 mmoles/l) (Fig. 5).

3.2. Analysis of blood lactate, glutathione and SOD during the performance of SK

We also looked for the changes in above parameters during SK performance. For this, besides the 0 time point, the blood samples were taken at 45 min and at 65 min post initiation of SK. The controls were just relaxing and were not practicing SK. Blood was collected at similar time points from the controls as for SK practitioners. We observed that there was statistically non-significant increase in glutathione levels at 45 min (303.8 ± 12.73 nmoles/mg protein, $t = -0.516$, $P = 0.618$) and at 65 min (413 ± 64 nmoles/mg protein, $t = -1.869$, $P = 0.095$) in practitioners whereas no

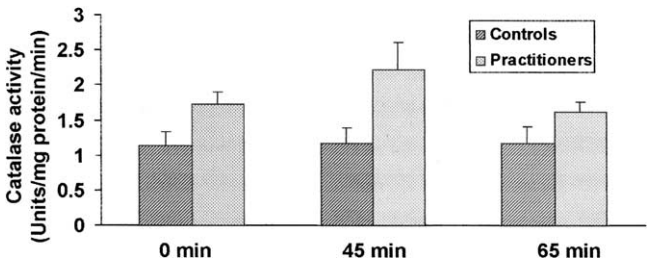

Fig. 3. Bar diagram showing the catalase activity (mean ± S.E.) in controls and in practitioners of SK at various time points during SK. The controls did not show a significant change. Note the higher basal level of catalase in long term practitioners of SK. Each experiment was done in duplicate.

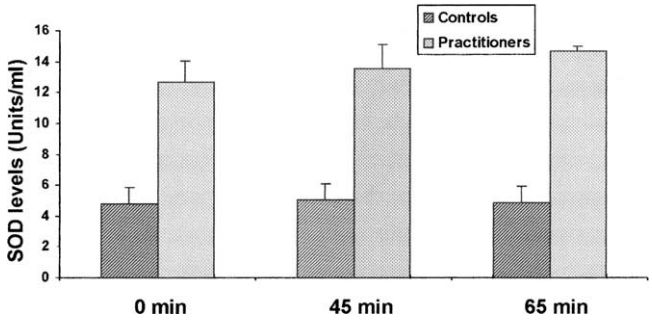

Fig. 4. Bar diagram showing the comparison of SOD activity (mean ± S.E.) in controls and in practitioners of SK at various time points of SK. The controls did not show a significant change. Note the higher basal level of SOD in long term practitioners of SK. Each experiment was done in duplicate.

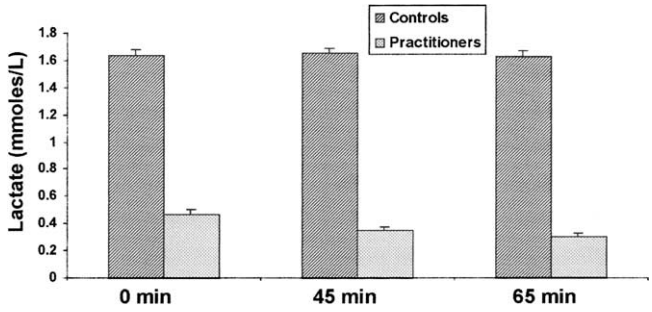

Fig. 5. Bar diagram showing the comparison of lactate levels (mean ± S.E.) in controls and in practitioners of SK at various time points during SK. The controls did not show a significant change. Note the lower basal level of lactate in long term practitioners of SK. Each experiment was done in triplicate.

significant changes were observed in the controls (Fig. 2). Similar results were obtained for catalase and SOD. A statistically non significant increase was seen for catalase at 45 min (2.218 ± 0.387 units/mg protein/min, $t = -0.613$, $P = 0.555$) of kriya and the increased levels returned to slightly less than the basal levels at 65 min (1.613 ± 0.152 units/mg protein/min, $t = 0.714$, $P = 0.491$) of the kriya (Fig. 3). A significant increase (19.37 ± 0.353 units/ml, $t = -5.301$, $P = 0.001$) in SOD was seen at 65 min of kriya, whereas the increase at 45 min of the kriya was not significant (13.586 ± 1.547 units/ml, $t = -0.422$, $P = 0.683$) (Fig. 4). No significant changes were observed either in catalase or in SOD in the case of controls.

A drop in blood lactate was observed at 45 min (0.348 ± 0.032 mmoles/l, $t = 3.072$, $P = 0.013$) and 65 min (0.3 ± 0.026 mmoles/l, $t = 4.123$, $P = 0.0006$) of Kriya, whereas there was negligible change in the blood lactate levels in the case of controls (Fig. 5).

However, we did not find any statistically significant correlation among these parameters or between these parameters and the anthropometric measurements of the individuals (Table 1).

Table 1
Mean±S.E. of the anthropometric measurements

Age (in years)	Height (in cm)	Weight (in kg)	Body surface area (sq. m.)
Mean±S.E. of the anthropometric measurements of the controls included in the study			
24.14±0.47	174.36±1.82	65.35±1.82	1.78±0.031
Mean±S.E. of the anthropometric measurements of the practitioners included in the study			
24.4±0.6	175.8±1.17	69.9±2.45	1.84±0.033

4. Discussion

There is growing evidence to suggest that oxidative stress or free radicals/ROS induce DNA damage and contribute to the patho-physiology of atherosclerosis, CHD and other chronic diseases associated with aging such as cancer and rheumatoid arthritis and cataract (Schneider et al., 1998). Many factors can contribute to the levels of oxidative DNA damage in cells (Adachi et al., 1993). Each individual is exposed to oxidants; with interindividual variation in antioxidant defenses, and DNA repair ability. All these factors together determine the extent of oxidant-induced DNA damage in each individual, and the levels of such damage may well contribute to cancer risk, especially in tissues in which other changes may have already occurred. Psychological stress has been shown to increase oxidative stress. Stress reduction with TM program has been shown to decrease psychosocial stress (Alexander et al., 1994). Epidemiological data (Alexander et al., 1994; Schneider et al., 2001) and controlled clinical trials (Zamarra et al., 1996) have suggested that lower rates of CHD morbidity and mortality are associated with long term practice of TM in older subjects. In addition rates of cancer and aging have also been reported to be lower in TM practitioners (Schneider et al., 2001). However, there are no studies available on the effect of stress reduction with SK practice on oxidative stress. Our findings suggest that persons who regularly exercise SK have a better antioxidant status as compared to non-practitioners.

During anxiety and tension there is a rise in the level of lactate in the blood. Blood lactate levels have been shown to be decreased during meditation compared with a pre-meditation control period in experienced meditator (Schneider et al., 2001). An increase in 4 h muscle blood flow, has also been reported (Jevning et al., 1992; Schneider et al., 2001) thus speeding oxygen delivery to muscles and reducing the need for anaerobic metabolism and consequent lactate production. In our study, we have seen a significant decrease in blood lactate levels at 45 and 65 min during kriya. The decrease in lactic acid concentration is indicative of general hypometabolism during meditation, and there may be a concomitant increase in metabolic activity in other regions of the body such as brain and skin, because they along with muscle tissue account for major portions of total cardiac output after renal and hepatic contribution have been subtracted.

In another study a marked decline in RBC glycolytic rate during meditation was seen and this decline significantly correlated with decreased plasma lactate

concentration and with relaxation (Jevning et al., 1992). The decrease in RBC metabolism is not an epiphenomena of respiratory change or of substrate availability because of the observed lack of variation in blood pH, gases, blood glucose and hematocrit during meditation. Cerebral blood flow is perhaps due to shunting of blood away from renal and hepatic circuits. By sparing glucose, red cell and muscle metabolic changes can also be integrated within the putative response by postulating that they provide extra glucose for central nervous tissue utilization. Red cell and nervous tissue have in common almost obligatory metabolic dependence upon glucose and together account for most of blood glucose consumption in normal persons. With respect to muscle, a shift for β oxidation of fatty acids would spare glucose and cease to generate carbon dioxide. Thus there is integrated regulation of metabolism during meditation to supply glucose for increased cerebral metabolism (Jevning et al., 1992).

To the best of our knowledge, this is the first study of its kind to look at the short and long term effects of SK on glutathione, catalase, SOD and blood lactate levels. Glutathione, catalase and SOD are antioxidant enzymes that protect against damage caused by free radicals. Our results indicate that persons who practice SK have increased levels of all the three, thus indicating a better overall antioxidant defence mechanism. As the link between psychosocial and oxidative stress is well established (Scarpellini et al., 1994; Adachi et al., 1993), our study highlights the fact that practicing SK regularly can lead to better stress regulation by improving the antioxidant defence mechanism. The fall in blood lactate levels during SK suggests a relaxation response, perhaps due to hypometabolic state leading to decreased plasma lactate levels by muscles and RBC. The blood glucose spared by the muscle and RBC may thus be available for utilization by the brain leading to mental alertness but bodily relaxation.

In conclusion, the findings of this exploratory study suggest that lower levels of blood lactate and a better antioxidant status in practitioners are associated with practice of SK technique. The police persons are under a lot of stress in India and if such stress management programs are incorporated in police training programs, it will help them in the long run. This technique may provide a mechanism for reducing incidence of CHD and improvements in other age-related and ROS associated disorders. Further research with a randomized clinical trial with a larger sample population is required to verify the impact of SK on antioxidant and lactate status.

Acknowledgements

This work was inspired by Sri Sri Ravi Shankarji and was carried out in collaboration with Ved Vignan Mahavidyapeeth, Bangalore, India. This work was funded by Central Council for Research in Yoga and Naturopathy (CCRYN) and we are grateful to the Art of Living, Delhi Chapter who helped in the organization of the study, and the Delhi Police Training College volunteers for providing their blood samples.

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