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ORIGINAL ARTICLE

The role of deep breathing on stress

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Abstract The objective of this study was to verify, in a sample of university students, whether a relaxing technique called deep breathing (stress Intervention Functional IFA) is capable to improve the mood and to reduce the levels of stress. Thirty-eight adult healthy subjects (aged between 18 and 28 years) volunteered the study. They were randomly divided in two groups, the Experimental Group ($N = 19$) and the Control Group ($N = 19$). The subjects of the Experimental Group were submitted, once per week, to 10 treatment's sessions of Anti-stress Protocol, each lasting 90 min, whereas subjects of the Control Group sat ten times for 90 min, once per week, without practicing any treatment. The psychological state of mood and stress was evaluated using Measurement of Psychological Stress (MSP) and Profile of Mood State (POMS), while the biological profile of the stress was detected by measuring the heart rate and the salivary cortisol. The results obtained from the present research support the possibility that deep breathing technique is capable to induce an effective improvement in mood and stress both in terms of self-reported evaluations (MPS and POMS) and of objective parameters, such as heart rate and salivary cortisol levels. No statistically significant difference was found between men and women.

Keywords Deep breathing · Stress · Cortisol · Emotion

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Introduction

Psychological stress is a major risk factor for the development and progression of a number of diseases, including cardiovascular disease, cancer, arthritis, and major depression [1].

A fact known by the scientific community is that emotions in general, and stress in particular, produce interrelated functional changes, mainly through the vegetative nervous system, the endocrine system and the immune system.

A protracted state of tension induces in the individual psychological, physiological and behavioral effects that over time can lead to harmful consequences [2].

Contemporary society maintains individuals in a constant struggle for success without taking into account their needs and how much all this costs them. For professional sportsmen, mood and level of stress have been always considered aspect they must learn to control. Moreover, it has reported an increase of alteration of mood states and of levels of stress in young students [3, 4].

The objective of this research is to verify, in a sample of university students, mood and stress levels, for evaluating the efficacy of a particular relaxing technique, called deep breathing (stress Intervention Functional IFA), for ameliorating the mood and for returning to normal levels of stress.

In Japan, deep breathing is widely used as a method of reducing tension and mood. It is a fundamental technique used in various relaxation methods and is also incorporated

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in qigong, yoga and progressive muscle relaxation [5]. However, in that study the evaluation of the effects of the different relaxation method on mood and stress was carried out using self-reported evaluations.

In this study, we investigated the effects of deep breathing on mood and stress not only with self-reported evaluations but also measuring objective parameters, as heart rate and salivary cortisol levels. The used self-reported psychological tests were the Measurement of Psychological Stress (MSP) and the Profile of Mood State (POMS), while the biological profile of the stress was evaluated by measuring heart rate and levels of salivary cortisol [6]. This study is intended as a preliminary evaluation for a larger intervention study.

Materials and methods

Subjects

Thirty-eight adult healthy subjects, students of the degree course in Psychology at the University of Catania, volunteered the study. Participants aged between 18 and 28 years and were randomly divided in two groups, with 19 subjects forming the Experimental Group and the other 19 representing the Control Group. Written informed consent was obtained from the subjects.

Protocol

The subjects of the Experimental Group (three males and 16 females with a mean age 23.2 years ± 2.74), were subjected, once per week, to ten treatment sessions of Anti-stress Protocol (see below), each lasting 90 min at the Functional Psychotherapy Center of Catania. The treatment took place between 9 and 11 a.m., and was always practiced by one of us (P.F.).

The subjects of the Control Group (four males and 15 females with a mean age 23.2 years ± 2.46), summoned ten times, once per week, at the Functional Psychotherapy Center of Catania, where they sat between 9 and 11 a.m. for 90 min without practicing any treatment.

At the beginning of the first session (I), fifth session (II) and tenth session (III) to all 38 participants were administered MSP and POMS, and was measured heart rate and salivary cortisone levels.

POMS test

To evaluate quantitatively the mood of the subjects they had to complete the 30-item Profile of Mood States (POMS) [7], which is a self-rating questionnaire consisting of six mood dimensions: Tension–Anxiety (T–A), Depression–Dejection (D), Anger–Hostility (A–H), Vigor (V), Fatigue (F), and Confusion (C). The total mood disturbance (TMD) score is calculated by subtracting the *V* score from the sum of scores for the other dimensions.

MSP test

To describe the variables able to provide a global index of the state of psychological stress, the Measurement of Psychological Stress (MSP) Scale (Measure du Stress Test Psychologique [8]) was used in the Italian translation and adaptation of Di Nuovo and Rispoli [9]. The MSP is a questionnaire consisting of 49 items based on different aspects related to the perception that the individual has of its condition (cognitive-affective, physiological, behavior). The choice of responses is made of a scale (Likert-type) whose possible answers are 1–4 (from "not at all" to "very").

Heart rate

Cacioppo and Berntson [10] demonstrated that an increase in heart rate often associate with the stressors, so we decided to use as a parameter for the evaluation of heart rate stress. The heart rate was measured in the Experimental as well as Control Group for 5 min at the beginning of the first session (I), fifth session (II) and tenth session (III). Measurement of heart rate started after that the subject sat for 15 min on a comfortable armchair.

Salivary cortisol

Levels of salivary cortisol were assessed using Radioimmunoassay Cortisol Test (RIA CT from RADIM), as previously described [11]. The saliva samples were collected three times in the same day (30 min after awakening and at 14:00 and 20:00 h; prior to breakfast, lunch and dinner, respectively). Saliva samples were collected by administering a tampon, chewed by the subject for about a minute; the samples were immediately frozen at −70 °C.

Prior to testing, participants were instructed regarding the purpose of the study and were asked to follow the instructions for the collection of saliva samples: avoid caffeine and acidic drinks, not brush teeth, eat or drink anything for 15 min prior to a sample collection.

Anti-stress protocol based on the psychological functional model

The anti-stress protocol is based on ten sessions and consists in the use of techniques designed to adapt the Functional Model proposed by Luciano Rispoli [12].

Table 1 illustrates the guidelines for the deep breathing technique that included.

Table 1 Guidelines for the deep breathing technique For more details of each individual technique, see Rispoli [12] and Blandini et al. [13]

Session	In orthostatic position, relaxing of the head and neck, rotating of shoulders, breathing deeply, yawning and imagining to follow the alternation of the breath
Session	Beat hands and feet, chilling down, staggering, imagining to do somersaults between the clouds, sharing
Session	Feel parts of the body (standing and walking), contacting back to back the others of the group, remembering tenderness, sharing
Session	In orthostatic position, moving the pelvis and modulating the voice, breathing as a butterfly, imagine smooth hills and a calm lake, sharing
Session	Contracting and releasing the body's muscles, the shoulders to lift and leave, raising and lowering the shoulders, imagining to breathe deeply, sharing
Session	Grimacing, exhaling with eyes half-closed and mouth open, yawning, crawling in the direction of the head, sharing
Session	Game with clothespins, breathing and moving the pelvis, imagining of getting lost, self-massaging, imagining to dive, sharing
Session	Groping in the dark with eyes closed, producing tremors and convulsions, imagining to follow the alternation of the breath, sharing
Session	Rolling on the others of the group, self-massaging the back, self-massaging the neck, imagining to be a leaf in the wind, sharing
Session	In orthostatic position, relaxing of the head and neck, rotating the shoulders, breathing deeply, moving together body and arms, imagining to fly, imagining sand dunes in the wind, sitting in a circle to greet

Statistical analysis

Data are presented as the mean ± standard deviation (SD). The slope of the diurnal change in cortisol level was calculated to estimate how each player fit the normal (i.e., descending) profile. A linear regression of the three cortisol values on the sampling times was calculated, with data pooled for each player. Steeper slopes represented smaller β coefficient values for the slope of the regression, indicating that the decrease in cortisol was more rapid. Flatter slopes (larger β coefficient values) indicated slower decreases or abnormally timed peaks. The average area under the curve (AUC) was calculated by trapezoidal estimation using cortisol values. The AUC 'with respect to ground' (AUCg) was also calculated [11] which provides information regarding the total hormonal output, and thus the basal activity of the Hypothalamic–pituitary–adrenal axis of the participants. Comparison were carried out using the unpaired Student's *t* test or one-way repeated-measures ANOVA (Friedman test) followed by Dunn's multiple comparison test. Correlation analysis was carried out using one-tailed Pearson's correlation analysis, and significance was set at $p < 0.05$. All analyses were performed using GraphPad Prism version 6.03 for Windows (GraphPad Software, San Diego, CA, USA) and were carried out according to the guidelines for reporting statistics in journals published by the American Physiological Society [14].

Results

POMS test

The scores of the six subscales of the POMS separately show significant differences in the group of deep breathing (stress intervention functional IFA), in different parameters, summarized by the total mood disturbance (TMD).

As can be seen in Fig. 1, the value of TMD shows a significant reduction ($p > 0.05$) in the Experimental Group between first (I) and last (III) session of deep breathing (stress Intervention Functional IFA). No significant changes were observed in the Control Group. No statistically significant difference was found between men and women in both Experimental and Control Group.

MSP test

As can be seen in Fig. 2, the values of MSP at the first session test in both groups show a medium level of stress, with values ranging between 90 and 100. The values obtained after the fifth and tenth session were unchanged in the control group (90 and 100). On the contrary, in the Experimental Group the values of MPS show a significant ($p > 0.05$) reduction in the levels of stress between the first and tenth session. No statistically significant difference was found between men and women in both Experimental and Control Group.

Heart rate

The heart rate were measured in the Experimental as well as Control Group for 5 min at the beginning of the first session (I), fifth session (II) and tenth session (III).

As can be observed in Fig. 3, the values obtained in the Control Group do not show any significant change induced by deep breathing, whereas in the Experimental Group the level of heart rate exhibits a significant ($p > 0.05$) reduction between the first and tenth session. No statistically significant difference was found between men and women in both Experimental and Control Group.

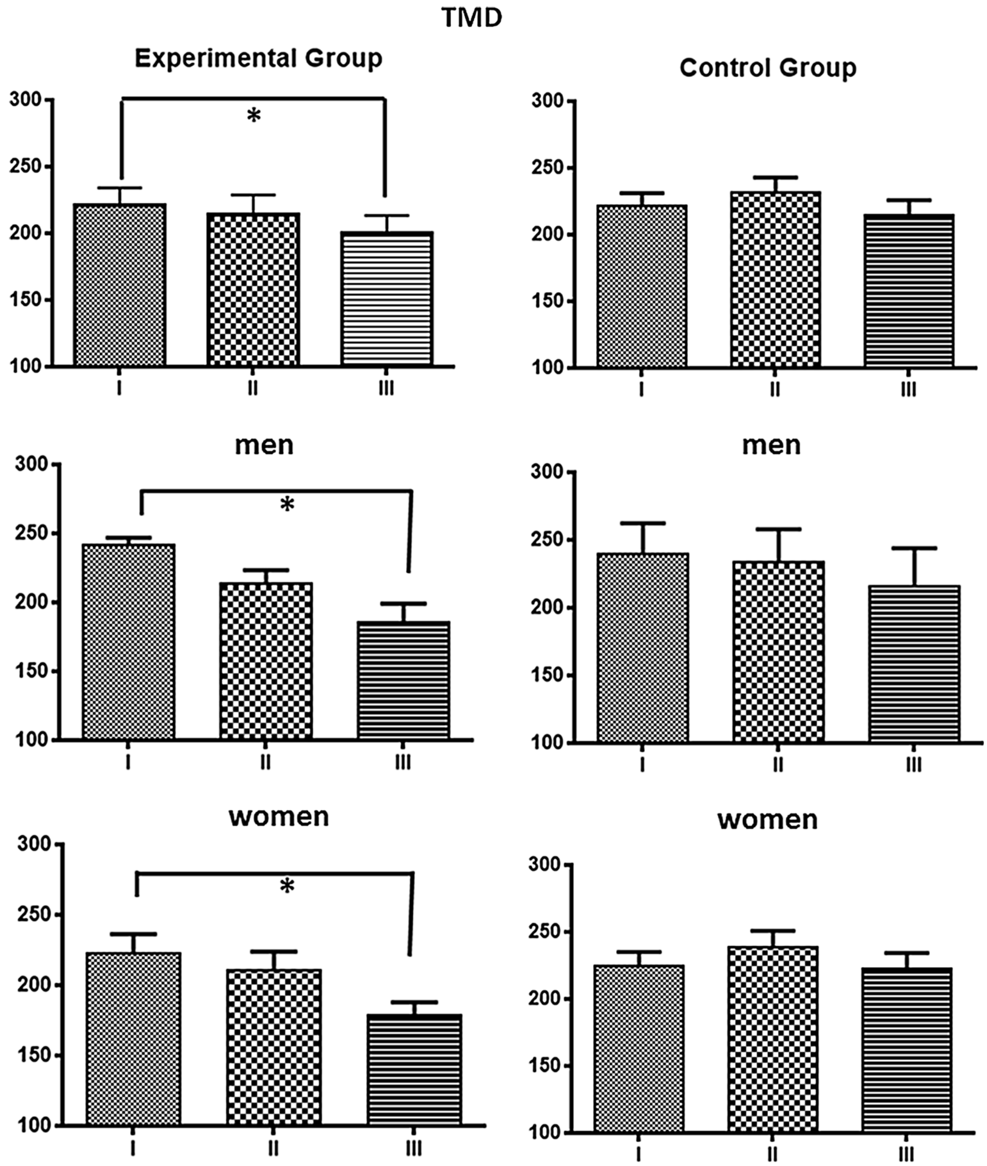

Fig. 1 Mean values of total mood disturbance (TMD) in the Experimental as well as Control Group, measured at the beginning of the first session (I), fifth session (II) and tenth session (III). In the *first row*, data of the entire sample are presented, while in the *second* and *third row* are shown separately data for men and women, respectively. Symbols from ANOVA with Dunns's multiple comparison test: *$p < 0.05$

Salivary cortisol

As can be seen in Table 2 and Fig. 4, the levels of salivary cortisol at the beginning of the first session show comparable values in Experimental and Control Groups. The showed AUCg values are obtained by averaging the levels measured 30 min after awakening, at 14:00 and 20:00 h. As can be seen in both Table 2 and Fig. 4, in the Control Group the three measurements did not exhibit significant differences. However, in the Experimental Group there is a statistically significant improvements ($p > 0.001$) between the first (I) and the last session (III), as well as between the fifth (II) and the last session (III, $p > 0.05$). No statistically significant difference was found between men and women in both Experimental and Control Group.

Discussion

It is generally believed that the deep breathing, practiced using various techniques, such as qigong and yoga, improves the sense of well being.

Scientific literature reports remarkable positive effects induced by the oldest relaxing technique, yoga [15], as improvement in motor abilities [16], heart rate, blood pressure and body composition [17, 18], cardiorespiratory function

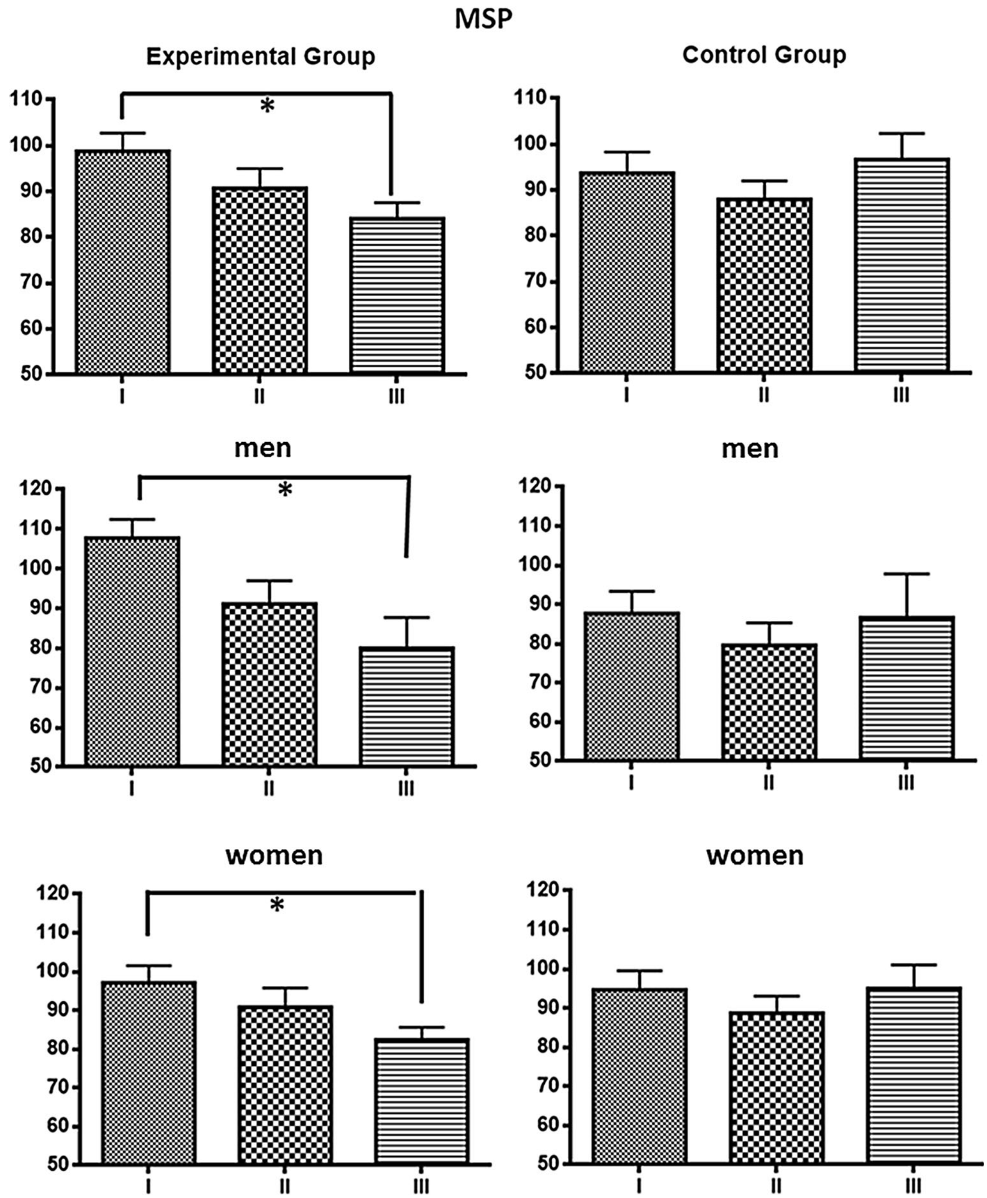

Fig. 2 Mean values of Measurement of Psychological Stress (MSP) in the Experimental as well as Control Group measured at the beginning of the first session (I), fifth session (II) and tenth session (III). In the *first row*, data of the entire sample are presented, while in the *second* and *third row* are shown separately data for men and women, respectively. Symbols from ANOVA with Dunns's multiple comparison test: $*p < 0.05$

[19], forced expiratory volume [18] and maximum vital capacity [20]. Positive effects were found also on mood states as anxiety [21] and perceived stress [22, 23]. The study carried out by Hayama and Inoue [5] showed a reduction of 'tension anxiety' and fatigue through the use of deep breathing.

In this study, the use of the technique of deep breathing by a group of university students showed a significant improvement between the beginning and the end of the training.

The improvements occurred both on the examined physiological effects (heart rate and salivary cortisol levels) and on mood and perceived stress, improvements not observed in the Control Group.

Comparable results were found by Paul et al. [24], who observed in a longitudinal study, conducted on a sample of students that the use of deep breathing is capable to reduce stress, allowing an improvement in academic performance. This has led the Authors to include in subsequent courses of this method.

Recently, a systematic review on development of stress in dental students, reported significant improvements with deep breathing [25].

Plasma lactate concentrations increase during acute psychosocial stress in humans [26] and in the last years it has been observed that the excitability of the Primary Motor Cortex is improved by high concentrations of blood lactate, induced not only with a stressful condition as an exhaustive exercise but also with an intravenous injection [27–31]. It is worth noting that repetitive magnetic stimulation of primary motor cortex is per se capable of increasing blood lactate [32]. On the other hand, an

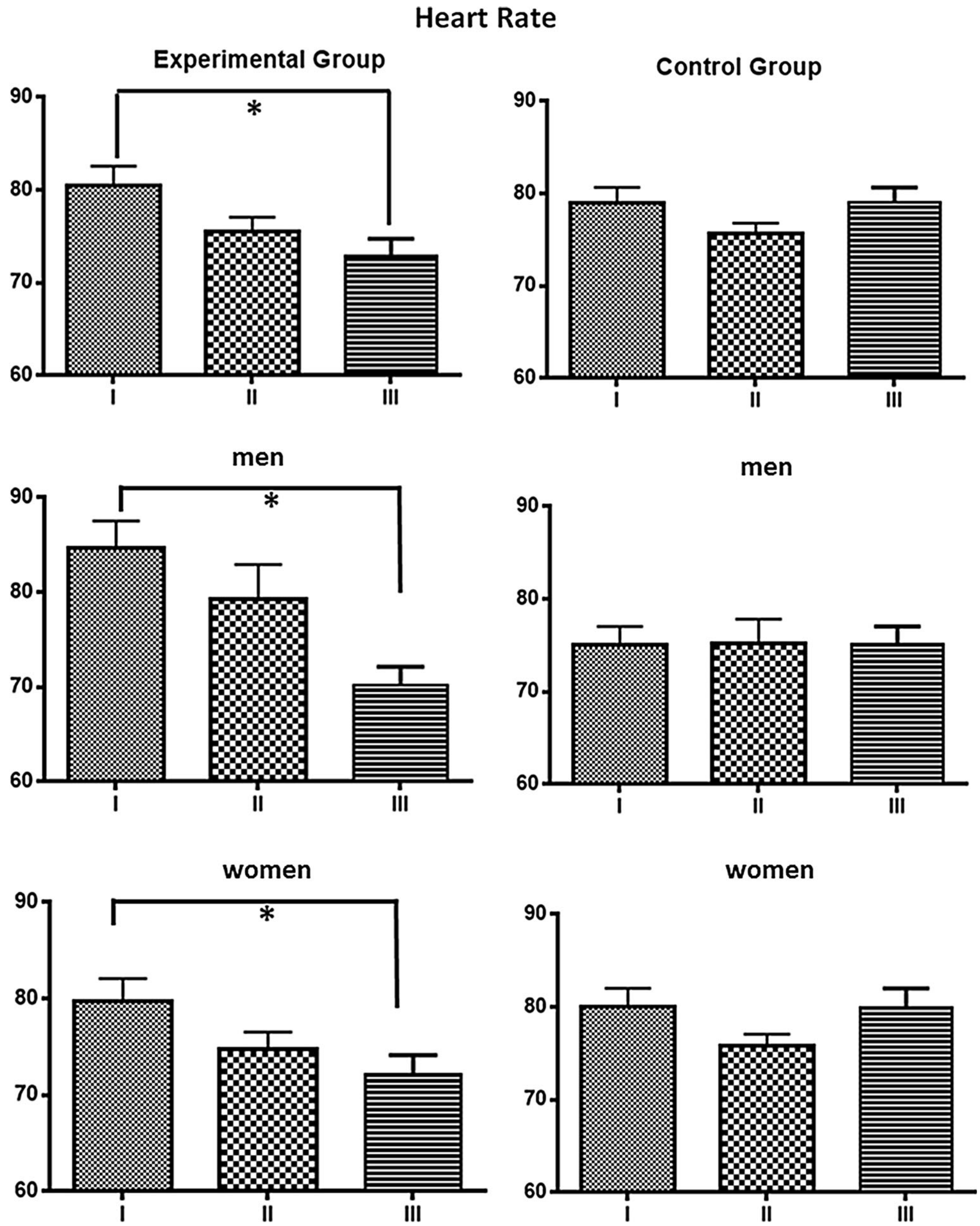

Fig. 3 Mean values of heart rate in the Experimental as well as Control Group measured at the beginning of the first session (I), fifth session (II) and tenth session (III). In the *first row*, data of the entire sample are presented, while in the *second* and *third row*, are shown separately data for men and women, respectively. Symbols from ANOVA with Dunns's multiple comparison test: $*p < 0.05$

Table 2 Mean levels (±standard deviation, SD) of salivary cortisol (pg/ml) in the Experimental Group and Control Group

	I	II	III
Experimental group			
Mean values	9.62	9.53	7.34
SD	1.34	1.53	2.18
Control group			
Mean values	9.55	9.53	9.58
SD	1.49	1.80	1.69

exhaustive exercise induces a reduction of the excitability of the Supplementary Motor Area [29] and a worsening of attentional processes [33, 34].

In conclusion, although this research have to be considered a pilot study, the use of deep breathing techniques has lead to an effective improvement in the management of stress in daily life, and therefore, could exert positive influences on the stress conditions that the student must face during the course of his/her studies.

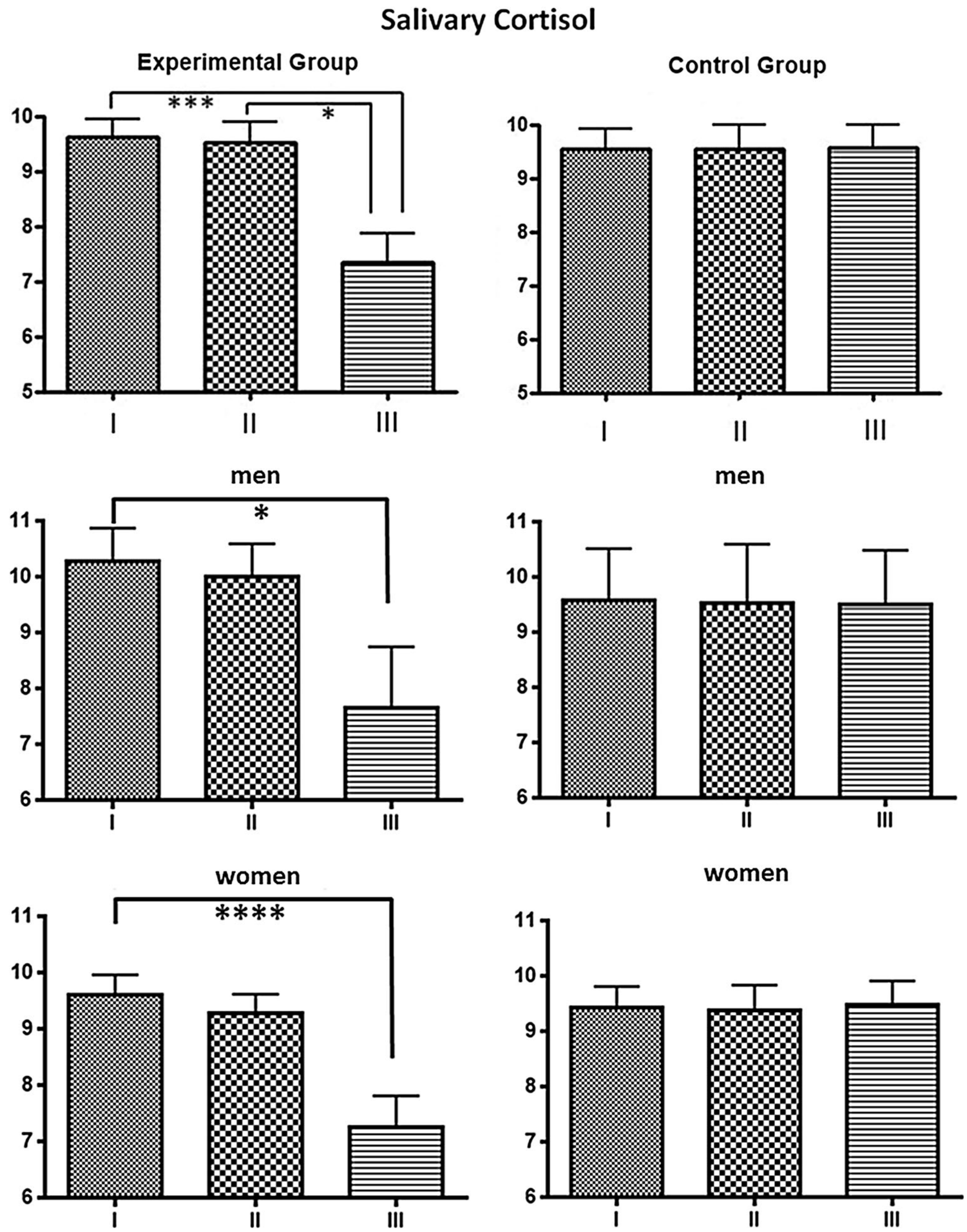

Fig. 4 Mean values of salivary cortisol (pg/ml) in the Experimental as well as Control Group measured at the beginning of the first session (I), fifth session (II) and tenth session (III). AUCg values of salivary cortisol were obtained by averaging the levels measured 30 min after awakening, at 14:00 and at 20:00 h. In the *first row*, data of the entire sample are presented, while in the *second* and *third row*, are shown separately data for men and women, respectively. Symbols from ANOVA with Dunns's multiple comparison test: *$p < 0.05$; ***$p < 0.001$

Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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