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PHOTOACOUSTIC SPECTROSCOPY FOR NON-INVASIVE ANALYSIS OF HUMAN RESPIRATION

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Abstract. Understanding the differences between the normal breathing and diaphragmatic breathing can have a significant impact on your endurance during exhaustive exercise. We used a photoacoustic laser spectrometer with CO_2 emission in the infrared range to investigate the physiological alterations in differences between diaphragmatic breath and normal breath. We monitored the exhaled ethylene breath of a 11 healthy women participants (including the coach) during normal respiration session and immediately after 50 minutes performing diaphragmatic breathing. The results demonstrate that diaphragmatic breathing decreases the level of oxidative stress after therapeutic exercises which suggest that the women's who practice this type of meditation could be protect from long-term adverse effects of exogenous free radicals.

Key words: diaphragmatic breathing, normal breathing, physiological alterations, photoacoustics.

1. INTRODUCTION

Diaphragmatic breathing is therapeutic and deeply relaxing, reduces stress, and is a fundamental procedure of Yoga meditation and other meditation practices. This way of meditation is the act of breathing deeply into the lungs by flexing the diaphragm rather than the rib cage [1].

Yoga breathing (pranayama) is an important part of health and spiritual practices in Indo-Tibetan traditions. Considered fundamental for the development of physical well-being, meditation, awareness, and enlightenment, it is both a form of meditation in itself and a preparation for deep meditation. Yoga breathing can rapidly bring the mind to the present moment and reduce stress [2].

Although exercise-induced reactive oxygen species (ROS) production can be produced via different pathways [3–6], we had suspected that diaphragmatic breathing could improve antioxidant defenses and therefore, decrease oxidative stress together with physiological alterations of the organism.

The question that arises is, could protect diaphragmatic breathing the subjects from long-term adverse effects of exogenous free radicals?

Because it is not possible to directly measure free radicals in the body, we approach this question by measuring the by-products (breath ethylene) that result from free radical reactions on the body's cell.

It is important to understand the risks and complications that may be associated with the procedure we are considering, because it is known that oxidative stress is quite common and often overlooked as a health risk with relaxation exercise or the breathing procedure (diaphragmatic *vs.* normal).

According to these features, our approach compared the ethylene biomarker produced as a by product of oxidative stress from the exhaled respiration of volunteers during normal breathing and after diaphragmatic breathing (Yoga meditation). To our knowledge, this is the first study which explore the effects of diaphragmatic breathing in Yoga procedure by determining the ethylene gas as a biomarker of oxidative stress caused by exhaustive physical activity.

2. MATERIALS AND METHOD

2.1. STUDY VOLUNTEERS

We selected 10 females practicing Yoga meditation (50 minutes/week) as a control group and 1 Yoga instructor with experienced to all levels of Yoga.

Both the control group, aged 28.7 ± 3.0 years, mean weight 56.8 ± 7.6 kg and mean height 161.5 ± 6.3 cm (± SD) and the coach, gave their informed consent prior to their inclusion (were informed of the purpose of the study).

None of the women's had taken antioxidants or medication supplements within the past 2 months that might alter the study outcome. Also the control women's were non-smokers, non-alcoholic, non-renal, non-diabetic and none of them had a history of medical or surgical events. On the day prior the test, products such as onions, leeks, eggs, and garlic were avoided.

Prior to the preservation of respiration, the women's were asked to avoid for at least 3 hours before or during the Yoga procedure: alcohol and coffee, food or beverages.

Before and after the Yoga procedure the women's drank only water to dehydrates. All of the collected samples were analyzed within 1 h after sampling over a period of 2 months. Yoga session was held in the evening between 20:00 and 20:50 pm.

2.2. STUDY PROTOCOL

We collected a clean respiration in 750 mL aluminum-coated bags (Quintron) and we analyzed the alveolar portion of the respiration for ethylene with a photoacoustic CO_2 laser spectrometer with emission in the infrared range (9–11 μm).

The photoacoustic CO_2 laser spectrometer used for the biomarker content measurement and presented in this report is described in other works by [7–10].

In brief, the technique utilizes a line-tunable CO_2 laser and a detector, where the gas (biomarker) is analyzed and detected.

Figure 1 shows a graph of the resonant frequency of the photoacoustic cell (detector).

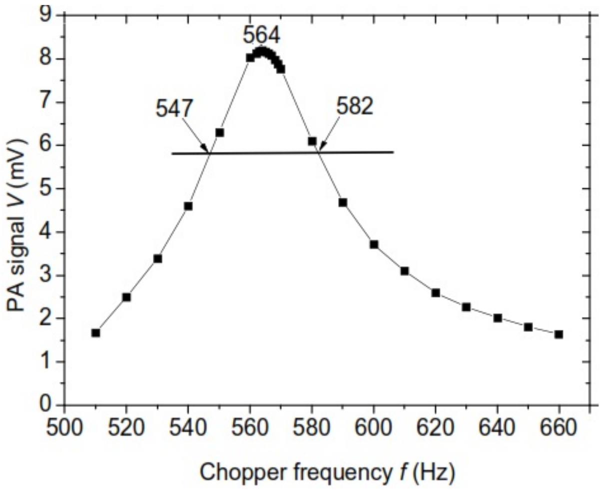

Fig. 1 – Resonance curve of the photoacoustic cell.

The acoustic resonator is characterized by the quality factor (*Q*), which is defined as the ratio of the resonance frequency to the frequency bandwidth between half power points. The amplitude of the microphone signal is $1/\sqrt{2}$ of the maximum amplitude at these points, because the energy of the standing wave is proportional to the square of the induced pressure.

The quality factor was measured by filling the photoacoustic cell with 1 ppmV of ethylene buffered in nitrogen at a total pressure of 1 atm and by tuning the modulation frequency in 10 Hz increments (2 Hz increments near the top of the curve) across the resonance profile to estimate the half width, as described above.

For this photoacoustic cell, the profile width at half intensity was 35 Hz, yielding a quality factor $Q = 16.1$ at a resonance frequency $f = 564$ Hz.

Figure 2 shows the calibration measurements (concentration dependent response) for ethylene, experimentally determined using commercially prepared, certified gas mixtures containing 0.96 ppmV ethylene diluted in pure nitrogen.

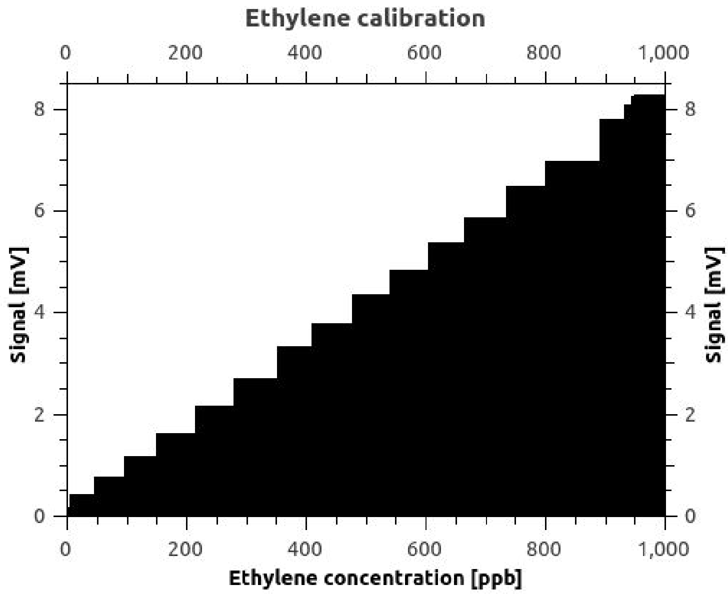

Fig. 2 – Calibration curve for ethylene.

For calibration we examined this reference mixture at a total pressure of approximately 1013 mbar and a temperature of 23°C, using the commonly accepted values: 30.4 $cm^{-1}atm^{-1}$ for ethylene [7, 8–11].

2.3. DETERMINATIONS OF BREATH ETHYLENE BIOMARKERS

The women's were required to exhale into the sampling bags first with a normal exhalation flow rate before the start of the Yoga procedure and then immediately after the 50 minutes training (20:00–20:50 pm) to relax by performing diaphragmatic breathing in a relaxing atmosphere.

To analyze the gas from the Quintron bags, we evacuate the extra gas and then we flushed the system with pure nitrogen at atmospheric pressure for few minutes and then the exhaled air sample can be transferred to the cell using a controlled flow rate of 600 standard cubic centimeters per minute.

An intensive cycle of nitrogen (purity 99.9999 %) washing was performed also between samples, in order to ensure the quality of each measurement. It has to be underlined that the measured photoacoustic signal is due mainly to the absorption of ethylene, but some traces of CO_2, H_2O, ethanol, etc., influence the measurements (overall contribution is less than 10%).

The response to all absorbing species at a given laser wavelength decreased considerably when we inserted a trap with a volume larger than 100 cm^3 (KOH pellets), proving that amounts of CO_2 and H_2O vapors in the breath can alter significantly the results, thus their removal being compulsory [7, 8, 12, 13].

3. RESULTS AND DISCUSSION

In this study, we compared the ethylene gas, as a by product of oxidative stress in normal breathing versus diaphragmatic breathing at 10 amateur women's who practicing yoga meditation and 1 experienced women in yoga procedure over a period of 2 months.

Figure 3 shows the average concentrations of breath ethylene for control group, before (normal breathing) and immediately after 50 minutes of diaphragmatic breathing.

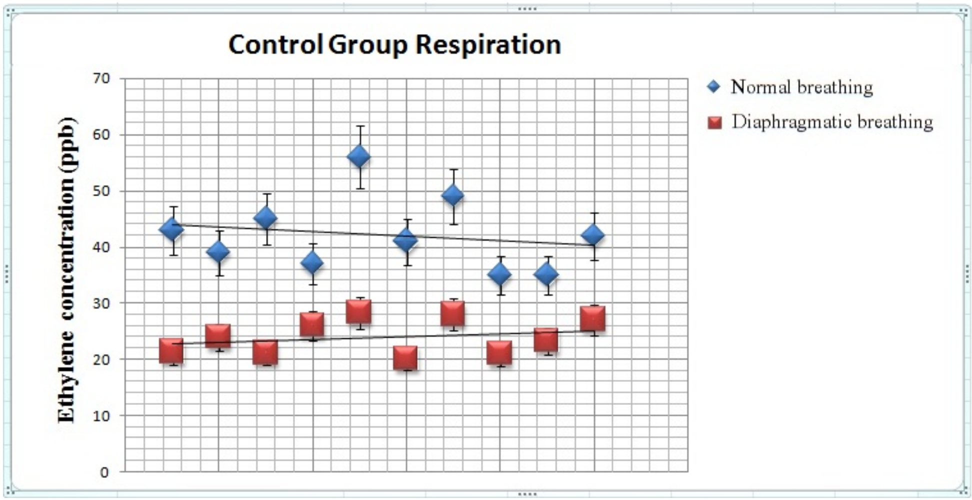

Fig. 3 – Control group respiration before (normal respiration) and after the Yoga procedure (diaphragmatic respiration); data shown are mean ± SD.

We see that the mean ethylene level from the respiration of the control group performing normal breathing is higher compared to the mean ethylene level from the respiration of the same control group but performing diaphragmatic breathing. The level of ethylene from the respiration was about 50% higher for normal breathing than for diaphragmatic breathing of the same control group.

Figure 4 shows the average concentrations of breath ethylene for the coach over a period of 5 week, before (normal breathing) and immediately after 50 minutes of diaphragmatic breathing (Yoga session).

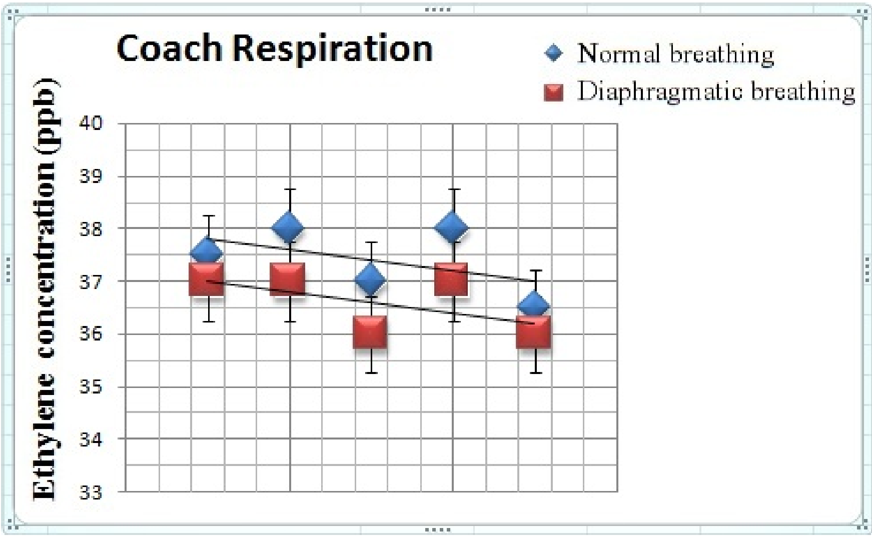

Fig. 4 – Coach respiration before (normal breathing) and after the Yoga procedure (diaphragmatic breathing); data shown are mean ± SD.

When we measure the ethylene from the exhaled breath of the coach we can observed that there is no significant change in the chemical levels of normal breathing compared with diaphragmatic ones. The differences were more evident for the control amateur group (Fig. 3), taking into consideration that the coach is an experimented instructor in Yoga meditation and in particular in diaphragmatic breathing and the respiration become accustomed to the feeling of the deep inhalation. This could be explained also as a body reaction to the decreased level of oxidative damage. The physiological basis of this hypothesis is still speculative and further work that would clearly identify the etiologic relation between breath ethylene biomarker and diaphragmatic breathing at Yoga instructors is required. Other possible confounding variables, such as age showed no statistically significant differences between the women's.

Oxidative stress seems to be a key piece in the relaxation induced by diaphragmatic breath. When oxidants exceed the antioxidant defence, biological systems suffer oxidative stress, with damage to biomolecules and functional impairment.

Most invasive measurements of oxidative stress in volunteers who practice relaxing breathing have been made on blood samples with help of d-ROMs test [14, 15] and reported a reduction in oxidative stress at diaphragmatic breathing, this therapeutic procedure being correlate with a lower oxidative stress levels. A new way to measure oxidative stress noninvasive in humans is to measure free radicals damage by analyzing early products of oxidation like exhaled hydrocarbons. Our determinations are based on the detection of biomarkers from breath (a non-invasive procedure) and are in good agreement with those (based on oxidative stress analysis) reported in the literature [14–18].

To our knowledge, this is the first study which explores the effect of diaphragmatic breathing versus normal breathing on the exhaled hydrocarbons (by products of oxidative stress) caused by exhaustive physical activity. Breath analysis is an emerging methodology that, being noninvasive and rapid, is ideally suited to clinical determination [18–21].

4. CONCLUSIONS

The use of related markers in exhaled breath air for normal versus diaphragmatic breathing analysis is theoretically reasonable; metabolic changes occur in the normal respiration that inevitably lead to the production of certain abnormal metabolites. These metabolites are transported through the blood to the alveoli of the lungs, through alveolar gas exchange and volatile metabolites will then be discharged into the air as components of each exhaled breath.

In the current study we analyzed breath ethylene produced as a by product of oxidative stress at women's who meditate indicated that diaphragmatic breathing from Yoga procedure correlate with lower oxidative stress levels together with a lower physiological alterations of the organism.

In conclusion, the results from this study support the hypothesis that relaxation induced by diaphragmatic breathing increases the antioxidant defense status in women's after exhaustive exercise.

Based on a non-invasive sampling method, stable in biological materials, and easy to measure, we conclude that photoacoustic CO_2 laser spectrometer analyses of breath ethylene from respiration appeared to distinguish normal from diaphragmatic breathing.

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The authors declare that they have no conflict of interest.

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