

Physiological responses during a single rebirthing (Breath work) session

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ABSTRACT

Objective: The present report aimed to look at the physiological responses during a typical single Rebirthing session.

Material and Methods: Ten healthy young women participated in the study. Their mean age, weight, and height were 37 ± 2.7 years, 54.1 ± 6.4 kg, and 161.2 ± 4.9 cm, respectively. The Rebirthing sessions took place at the Israeli Rebirthing Center in Tel-Aviv. The treatments were carried out by a qualified Rebirthing therapist that has experienced thousands of rebirthing sessions during the last 15 years. Sessions were performed in a dark, quiet room and executed in a one-to-one set-up with the same trained therapist. After around 40-50 minutes, the session approached its end. Metabolic, cardiovascular, pulmonary, and gas-exchange variables were measured breath-by-breath using a commercial portable metabolic system. All data were transmitted wirelessly to an adjacent room in the clinic and continuously monitored by the study's chief researcher.

Results: The primary study findings demonstrated that a typical Rebirthing session involving long (45-50 minutes) voluntary hyperventilation generated VO_2 , RER, HR, and O_2 pulse matching well with the physiological demands of the procedure (breath work). At the same time, the ventilatory-related responses exhibited, as expected, exaggerated outcomes, illustrated by the high session's peak and average values of the depth (tidal volume), breathing frequency, and minute ventilation. Gas-exchange attributes showed extremely shallow end-tidal CO_2 levels, high end-tidal O_2 , high respiratory exchange ratio, and very high levels of O_2 and CO_2 ventilatory equivalents. No significant grievances regarding participants' physical and mental/emotional feelings were reported in the present study.

Conclusions: The present study could not solve the apparent divergence between the observed (acute) physiological responses (mainly severe Hypocapnia) and the subjective participants' pleasant emotional state, and in many cases, spiritually uplifting, at the end of each treatment session.

Keywords: Rebirthing therapy, voluntary hyperventilation, Hypocapnia, body posture

INTRODUCTION

Conscious breathing practices for physical, psychological, emotional, and spiritual healing have a long and extremely varied history. Working with the breath in psychotherapy and other clinical disciplines also enjoys a long and rich history (1, 2), with the assumption that breathwork can resolve psychological pain, soften character armor, release tension in the body and create a sense of embodiment and equanimity (3, 4, 5).

In the early '70s, Leonard Orr created the technique known as Rebirthing (also known as Breastwork, Conscious breathing, Circular breathing, and Connected breathing).

According to numerous reports, predominantly subjective participants who underwent the Rebirthing treatment felt relieved from the stress of daily life and cured of several sicknesses that often stem from weakening our emotional state (e.g., 6, 7, 8).

The primary rationale for such positive effects of the Rebirthing therapy is based on the claims that an intense breathing process can improve focus and sleep quality, battle fatigue and increase energy (6, 9, 10).

Millions of rebirthing advocates worldwide claim that rebirthing treatment helps overcome physical and mental difficulties and improves overall well-being. These subjective reports imply improvement and healing, especially in the following physiological and emotional aspects:

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Mood improvement, lung capacity increase, physical fitness improvement, recovery from various respiratory problems, increase in energy levels throughout the day, recovery from a migraine and headaches, relief and recovery from chronic pain in different body areas, focus enhancement, and better learning ability (6, 7, 8).

The Rebirthing technique is simple and includes deep breathing through an open mouth, pausing between inhaling and exhaling, and vice versa. The process is usually performed lying down on a mattress with eyes shut. Breathing is similar to that during exercise or hyperventilation. However, it is meant to be slower and deeper, and the rhythm changes according to the patient experience before and during treatment.

When performed at upright body position, over-breathing or voluntary hyperventilation causes arterial blood CO₂ levels to abnormally drop, blood vessels in the body and brain to contract, the blood supply to the muscles and brain decreases, and their activity is impaired (11, 12). Prolonged hyperventilation (over several minutes) can cause several medical conditions, some of which are risky (13, 14). A partial list of typical physical phenomena seen during acute and extended hyperventilation includes confusion, palpitations, tetanic limb contractions (hands and or feet), paresthesia (primarily hands), feeling of suffocation, and, in severe cases, even fainting (13, 14).

To the best of our knowledge, not a single study has been published that examined the physiological nature, efficiency, effects, and physiological consequences of the Rebirthing treatment technique.

Considering the notable absence of sufficient scientific research and objective quantitative documentation of this challenging issue, and considering the impressive popularity of the Rebirthing therapy worldwide (approximately 20,000,000 participants in the past 40 years) (10), we conducted a controlled scientific investigation to examine both, the physiological responses during a single typical Rebirthing session and the effects a series of Rebirthing sessions on physiological and mental/emotional attributes, in healthy young women.

This manuscript reports the project's first part on the physiological responses observed during a single Rebirthing session.

MATERIAL and METHODS

Participants: Participants were recruited by the Israeli Center for Conscious Breathing from a pool of potential clientele.

Of all potential subjects, eleven healthy young women were selected, all from the central region of Israel. Their mean age, weight, and height were 37 ± 2.7 years, 54.1 ± 6.4 kg, and 161.2 ± 4.9 cm, respectively. All participants underwent a medical examination (including resting and exercise ECG) and were found healthy, and did not take any medications (except birth control pills). Participants were instructed to consume their regular diet, refrain from consuming caffeine or alcohol on the day before the test, and not to engage in intense exercise 24 h before the experiment; they were also instructed to refrain from eating at least two hours before the laboratory visit.

The Helsinki Committee for the Protection of Human Subjects (Institutional Review Board) of the Kaplan Medical Center in Rehovot, Israel, approved all study protocols and procedures. The subjects were fully informed of the procedures, risks, and discomforts in participating in the study, and all gave their signed, informed consent for participation. It was highlighted that participants have the right to withdraw from the study at any time and without any explanation.

Out of the eleven "starters," only one participant withdrew from the study (due to early pregnancy).

Study setting

Preliminary visit: Preliminary measurements for this study were taken at the Human Performance Laboratory at Washington Hill College in Israel.

During the first visit, the participants received a detailed explanation of the study aims and procedures and signed an informed consent form. The participants provided information on their medical history and physical activity habits. Weight and height were measured to the nearest 0.02 kg and 0.1 cm, respectively, using a Shekel model H151-8 scale (Shekel Scales Ltd., Kibbutz Beit Keshet, Israel), while the participants were barefoot and wearing light exercise clothing.

All participants performed the resting Pulmonary Function Test (PFT) (COSMED K4b2, Rome, Italy) to ensure healthy and normal lung function. The tests were carried out according to the ATS guidelines (15).

During this visit, participants were introduced to a short (10 min) demo Rebirthing session using the actual study's apparatus to minimize learning and habituation effects.

The Rebirthing session

Location - The Rebirthing sessions occurred at the Israeli Rebirthing Center in Tel-Aviv. The treatments were carried out by a qualified rebirthing therapist (the same in all sessions) who has experienced thousands of rebirthing sessions during the last 15 years.

Sessions were performed in a dark, quiet room and were executed in a one-to-one set-up (see picture) with the same trained therapist for all.



Rebirthing (Breath-work) Session – Participants were asked to remove their shoes and coat and lie down on a mattress during the Rebirthing session. The therapist then drapes a

blanket over the patient to help her stay warm and comfortable throughout the session. The therapist then guided the patient to start the connected breathing and build it up to a full, strong, and steady rhythm, pulling high on the inhale and releasing freely on the exhale.

After around 40–50 minutes, the session approached its end. In some participants, the breathing felt difficult, or sleepy, at which time the therapist's job was to recognize such signs and coach the patient through them. At the end of the session, the therapist gently escorted the patient back to the clinic's session.

Measurements taken during the Rebirthing session – Physiological measures were taken continually from the beginning to the end.

Metabolic, cardiovascular, pulmonary, and gas-exchange variables were measured breath-by-breath using a commercial portable metabolic system (COSMED K4b2, Rome, Italy) (see picture above). The metabolic system was calibrated for airflow and gas concentrations before each session, and the system accuracy was checked periodically using a metabolic simulator. A face mask (Hans Rudolph, USA) was used with resistance to breathing up to 10-mm water for air volume up to 200 liters/min.

The heart rate (HR) was recorded electronically (COSMED K4b2, Rome, Italy). The K4b2 gas analyzer has been previously validated and used in studies with varying activities and locomotion (16).

The breath-by-breath data were interpolated to 1s intervals, and the averages of uniform 10-s bins were used for graphical purposes and further calculations.

For safety and quality control purposes, all data were transmitted wirelessly to an adjacent room in the clinic and continuously monitored by the study's chief researcher.

Statistical analysis and calculations –Basic anthropometric characteristics were presented as averages and standard deviations (SD). Physiological data were calculated and presented as averages and standard error of the means (SEM) during the Rebirthing session.

RESULTS

Table 1 presents the group means (\pm SDs) of the PFT results performed during the preliminary visit. All ten female participants' PFT values (means \pm SD) were within the normal range, reassuring healthy participants.

Physiological responses during a single Rebirthing session – **Figure 1 (A, B, C, D, and E)** presents the metabolic (VO_2 , VCO_2 , RER)- and cardiovascular-related responses (HR, O_2 pulse) of the measured parameters.

The kinetic (trending phenomena) of oxygen consumption (VO_2) during the single Rebirthing session was relatively stable at 300–320 ml/min (see **fig. 1A**). An abrupt increase and decrease in VO_2 were observed only during the first and last 6–9 minutes of the session, respectively.

Unlike the relatively stable response of the VO_2 , the other two measured metabolic-related parameters, VCO_2 and RER, showed mild elevation during the first 7–8 min of the session,

followed by a continuous and linear decrease in values until the session-end (see **fig. 1B** and **1C**).

While VO_2 showed values slightly higher than typical resting VO_2 values for a population similar to the study's participants (young, healthy sedentary women), the level of alveolar CO_2 dropped continually throughout the session (except during the first 5 min), revealing persistently lower than resting pulmonary CO_2 values (see **fig 1-B**).

VO_2 - Oxygen uptake; VCO_2 - Carbon dioxide production/elimination; RER - Respiratory exchange ratio; HR - Heart rate; O_2 pulse - Oxygen pulse;

Consequential to the above VO_2 and VCO_2 responses, the calculated RER values from the 5th minute on demonstrating a continued reduction of the RER values, resulting from the changing proportions between the CO_2 removal (increases) and the O_2 consumption (constant) during the Rebirthing session (see **fig. 1C**).

Figures 1E and 1D present the responses (means \pm SEM) of the measured cardiovascular-related variables (HR and O_2 pulse) during the Rebirthing session. Both HR and O_2 pulse showed a relatively stable level throughout the Rebirthing session with the group mean values of 82.3 ± 18.2 b/min and 3.8 ± 0.93 ml O_2 /HR/min/kg, respectively. These values fit well with the imposed metabolic demands of the Rebirthing maneuver's energy requirements.

Vt , tidal volume; Bf , breathing frequency; VE , Minute ventilation; Vd/Vt , Estimated physiologic dead space; VE/VO_2 and VE/VCO_2 , Ventilatory equivalents for O_2 and CO_2 ; PETO_2 , End-tidal O_2 ; PETCO_2 , End-tidal CO_2 .

Figure 2 presents the response kinetics of the selected ventilatory- (Bf , Vt , VE) and gas-exchange-related variables (VE/VO_2 , VE/VCO_2 , PETO_2 , PETCO_2 , and Vd/Vt) during the Rebirthing session (Means \pm SEM).

Reviewing **figure 2** shows a gradual and continuous increase in breathing frequency (Bf - **fig. 2B**) throughout the treatment, from about 25 breaths/minute at the session starts approximately 65 breaths/min during the final stages of the session (sessions mean 54.8 ± 21.4 breaths/min). Parallel with the Bf , but in the opposite direction, there was a reduction in the depth of breathing (Vt - **fig. 2A**) from approximately 1.0 liter/breath in the early stage of the treatment to values around 0.5 l/breath during the last few minutes of the treatment (session's mean 0.67 ± 0.36 l/breath). Consequent to the opposing response patterns of the two breathing-related attributes (Bf and Vt), minute ventilation (VE - **fig. 2C**) was relatively stable throughout the session, varying between 25 and 30 l/min (session mean 27.6 ± 8.6 l/min), with an clear swing in VE during the final 10 min of the session (a decrease following by similar increase). Considering the significant increase in estimated physiological dead-space (Vd/Vt - **fig. 2D**) during the session, the observed ventilation pattern suggests a progressive reduction in alveolar ventilation (VA) during the Rebirthing session (17, 18).

The response profile of the ventilatory equivalent for oxygen (VE/VO_2 - **fig. 2E**), signifying the ventilatory efficiency (volume of ventilated air per one liter of oxygen consumed), showed an extremely high and relatively stable response throughout the session, varying between 90 and 100 l/l

(session mean 95.9 ± 21.1 l/l), with clear short increase and a decrease only at the start and the end of the session, respectively (**fig. 2E**).

A similar trending phenomenon of the respiratory equivalent for CO_2 (VE/VCO_2), representing the ventilation-perfusion ratio (V/Q), was also extremely high from the start to the end of the session (see **fig. 2F**).

In the three additional gas-exchange-related attributes measured in this study (PETO_2 , PETCO_2 , Vd/Vt), abnormal responses were noticed. While the PETO_2 values, indirectly inferring levels of PaO_2 (Wasserman et al. 2005; Inbar et al. 1994), are relatively high stable throughout the treatment session (~ 135 mmHg) (typical resting values 90 -120 mmHg) (see **fig. 2G**),

PETCO_2 values showed a sharp drop during the first 10 min of the session with relatively stable but exceedingly shallow values thereafter, until the session end (session group means of 14.5 ± 2.6 mmHg) (see **fig. 2H**) (typical resting values 35–45 mmHg). Such low values indicate substantial Hypocapnia and respiratory alkalosis from the start to the end of the Rebirthing session.

All of those mentioned above ventilatory- and gas-exchange-related response dynamics confirm sustained and severe Hypocapnia and respiratory alkalosis, owing to the voluntary hyperventilation (breathwork) during the Rebirthing session.

Table 1: Resting pulmonary function (PFT) (group means \pm SD) compared to relevant norms [% of predicted (predicted value)].

Parameter	Mean	SD	% pred.
FVC, Liter	3.65	0.035	110 (3.31)
FEV1, L/sec	3.07	0.26	93 (2.86)
FEV1/FVC, %	84.3	4.67	101 (85.2)
PEF, L/sec	7.05	0.55	94 (6.66)
MEF25-75, L/sec	3.53	0.81	105 (3.71)

FVC, forced vital capacity; FEV1, Force expiratory volume in 1 second; FEV1/FVC, %, The ratio between FEV1 and FVC; PEF, Peak expiratory flow rate; MEF25-75 %, Mid-expiratory flow rate; %pred., percentage of predicted normal (based on ECCS norms (16).

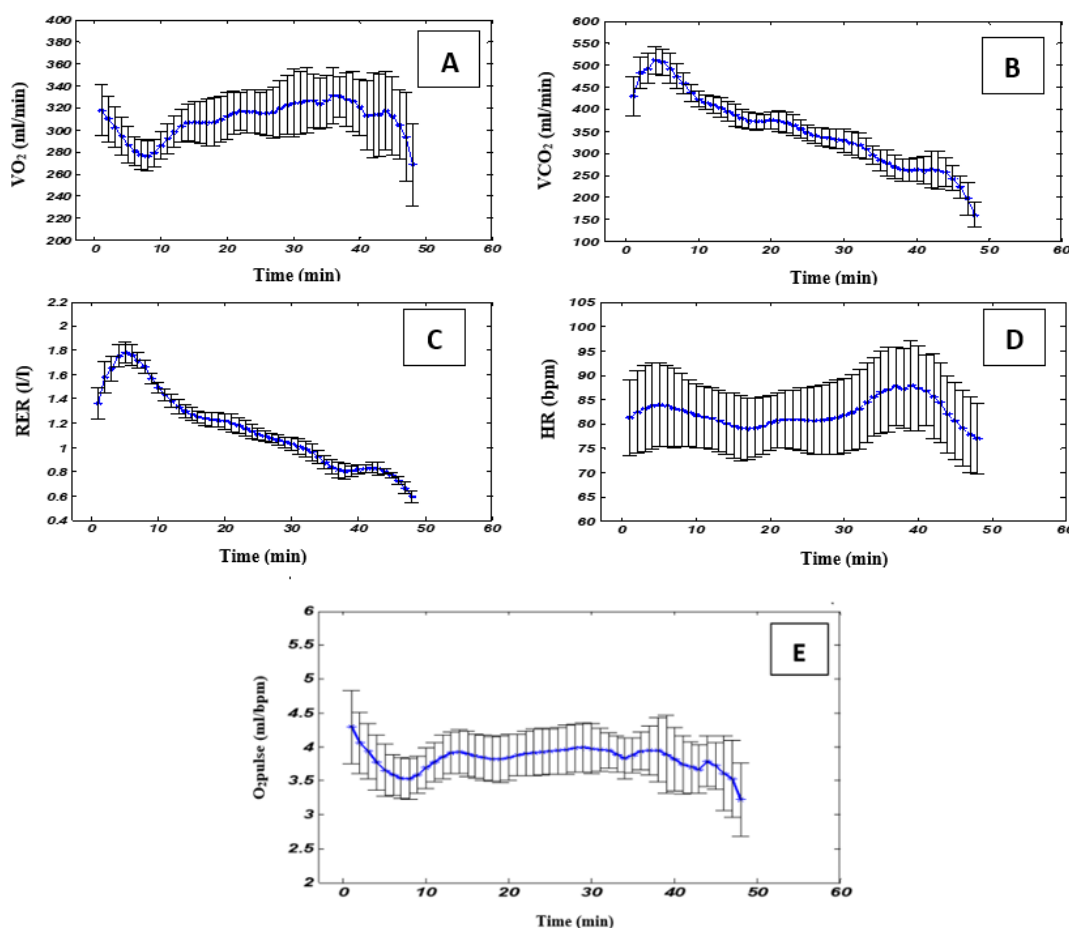


Figure 1: The response pattern of the metabolic- and cardiovascular-related parameters measured during the Rebirthing session (mean \pm SEM). VO_2 - Oxygen uptake; VCO_2 - Carbon dioxide production/elimination; RER - Respiratory exchange ratio; HR - Heart rate; Opulse - Oxygen pulse;

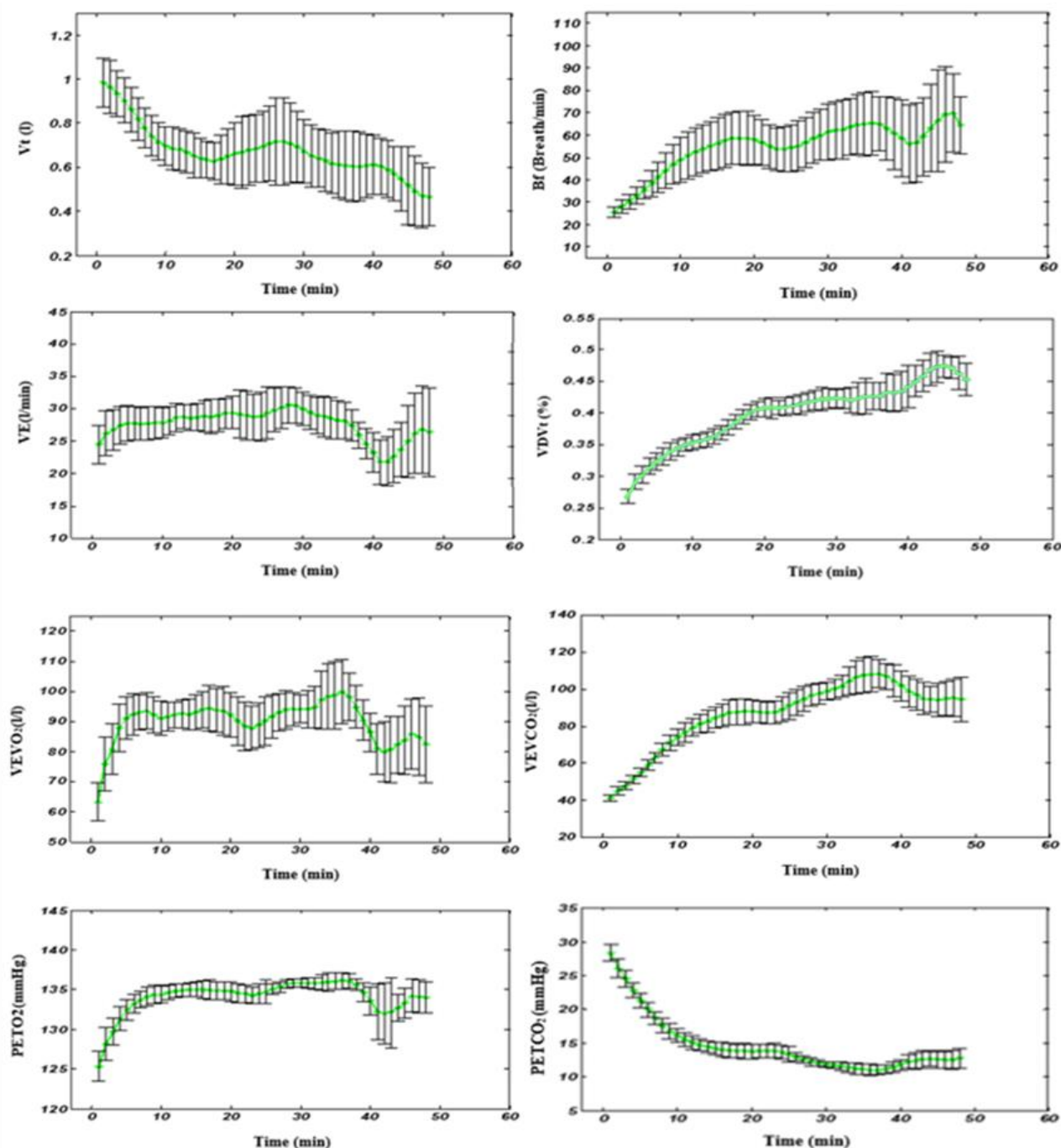


Figure 2: Response patterns of the ventilatory- and gas-exchange-related variables measured during the Rebirthing session (mean \pm SEM). Vt, tidal volume; Bf, breathing frequency; VE, Minute ventilation; Vd/Vt, Estimated physiologic dead space; VE/VO₂ and VE/VCO₂, Ventilatory equivalents for O₂ and CO₂; PETO₂, End-tidal O₂; PETCO₂, End-tidal CO₂.

DISCUSSION

Millions of rebirthing advocates worldwide claim that rebirthing therapy helps overcome physical and mental difficulties and improves overall well-being.

This study examined and defined the physiological responses observed during a single Rebirthing session in apparently healthy young women. In general, rebirthing therapy is given in a package, usually of 10–20 sessions.

The main findings of this study corroborate true voluntary hyperventilation (VHV) throughout the Rebirthing session.

True physiologic voluntary hyperventilation (VHV) was implied on the basis of the following measured physiological responses: extremely high ventilatory equivalents (VE/VO₂ and VE/VCO₂) and high and low PETO₂ and PETCO₂, respectively, throughout a substantial portion of the Rebirthing session (see figure 2).

Furthermore, while the metabolic- and cardiovascular-related responses during the treatment sessions (VO_2 , RER, HR, O_2 pulse – see **fig. 1**) match well with the physiological demands of the procedure (breath work), the ventilatory-related responses exhibited exaggerated outcomes, as illustrated by the relatively high session's peak and average values of the depth (Vt), frequency (Bf), and volume (VE) of breathing (see **fig 2**).

In this study, the heightened activation of the respiratory muscles throughout the therapeutic session caused a relatively stable level of oxygen uptake (VO_2) during the session's 40–50 min (session average 320 ± 45 ml/min) (see **fig. 1A**). Since the energy cost of breathing is relatively low (19), it is expected that even with 2 to 3-time resting minute ventilation, the whole-body O_2 consumption will not increase significantly.

As expected from such breathing maneuver, alveolar carbon dioxide levels (VCO_2) showed relatively high values during the first 5 min of the session, followed by a continued and linear drop of the session end (>3-fold drop) (session average 375 ± 53 ml/min) (see **fig. 1B**). An early increase in expired CO_2 was reported previously, suggesting some compensatory mechanisms that help limit the loss of CO_2 from the body's CO_2 stores (20, 21), thereby slowing down the alkalotic development.

The exceptionally high VE/VO_2 and VE/VCO_2 values suggest inefficient ventilation and pulmonary gas exchange. Typical (healthy) VE/VO_2 values at rest and maximal effort are 30 and 45 l/l, respectively. The respective typical values for VE/VCO_2 are 25 and 40 l/l, respectively (17, 18). Higher than normal resting or exercise values of VE/VO_2 or VE/VCO_2 are typically seen in patients with a gas-exchange abnormality, either at the lungs or at the local (muscle) level, and are often seen in patients being affected by either chronic obstructive lung diseases (COPD) or congestive heart failure (CHF) (17, 21).

Notwithstanding, the heightened ventilatory responses were an obligatory part of the Rebirthing procedure and were voluntarily executed by all participants.

Voluntary hyperventilation is used to expand the boundaries of human adaptation in sports medicine, occupational physiology, aviation, and space medicine (22, 23). The role of hyperventilation in all these conditions is to increase alveolar ventilation and blood oxygen saturation (24). Yet, this is frequently accompanied by several adverse effects, mainly: hypercapnia and alkalosis. However, under conditions of intense physical activity, reduction of partial oxygen pressure in the inhaled air, or the combined effect of these factors, the adverse impact of hyperventilation is largely compensated (17).

Abundant studies have been devoted to studying breathing mechanisms during prolonged voluntary hyperventilation (VHT), mainly during exposure to high-altitude hypoxia or load hypoxia and physical exercise (24, 25). Reported data on this topic (voluntary hyperventilation) is usually limited to only a few minutes of the exposure to hyperventilation (11, 13, 23). However, prolonged hyperventilation in the absence of intense physical activity and under normal O_2 partial pressure in the inhaled air in healthy humans remains

virtually unexplored [we found only a single published study that matched these criteria (20)].

In view of the above, a series of questions arise, the foremost being how could all study participants (and millions of the Rebirthing enthusiasts) "survive" the Rebirthing treatments without reporting any well-known hyperventilation-related adverse effect?

On the one hand, we have overwhelming popularity and positive reports on the effects of rebirthing therapy. However, hyperventilation under "resting" conditions could cause some severe adverse physical and or mental/emotional outcomes.

Considering the notable absence of sufficient scientific research and objective quantitative documentation on the above challenging issue, and in trying to answer this dilemma, the following are some possible tentative considerations/explanations:

One conceivable direction in resolving this puzzling issue (prolong VHV without any adverse effects) could be the body position at which the Rebirthing procedure is being conducted, i.e., recumbent.

It is well known that the loss of consciousness (syncope) results, among other causes, from cerebral hypo-perfusion (26). In an upright position, hypo-perfusion can occur when there is a significant decrease in the amount of blood reaching the heart or the inability of the heart to produce the pressure needed for transporting a sufficient amount of blood to the brain. When lying down, the hydrostatic pressure in the heart and circulation is much lower than during an upright position. Consequently, the ability to eject blood into the brain and other organs requires less energy. Thus, lying supine increases left ventricular filling pressure compared to upright posture (27, 28), causing substantial increases in end-diastolic and stroke volumes in healthy adults (29, 30). Further, Thadani and Parker (28) noted lower heart rates and higher left ventricular filling pressures, stroke indexes, and cardiac indexes in the recumbent position compared with an upright posture.

The above beneficial manifestations of lying prone, similar to those during Rebirthing treatment, may cause, among other changes, an increase in local perfusion pressure, increase in cardiac output, and an increase in cerebral blood flow, despite the partial constrictor effect of the induced hypercapnia on the peripheral and the cerebral vessels, thereby lessening cerebral ischemia/hypoxia (31, 32).

All or some of those mentioned posture-related (lying prone) positive physiological manifestations may, at least, partially explain the unexpected positive outcome of the Rebirthing therapy.

Another rationalization of the unanticipated outcomes of Rebirthing's VHT could be the notion that Hypocapnia is not the solitary cause of cerebral vascular tone. Tercero et al., (32) and Favre et al., (33) showed that cerebral blood flow (CBF) was normal during chronic Hypocapnia, suggesting that CO_2 does not alter the cerebral vascular tone. Kontos et al. (34) probably offered the best evidence that pH rather than CO_2 is the controlling messenger for CO_2 -mediated alterations of cerebral vascular tone. By applying artificial cerebrospinal fluid (CSF) topically to the cerebral cortex of

anesthetized cats, they showed that the diameter of cerebral arterioles responded only to changes in pH, regardless of fluid PCO_2 .

The above contentions could partially explain defeating the known adverse effects of prolonged voluntary hyperventilation and the positive and pleasant outmoded reported by all study participants and millions of advocates of the rebirthing technique worldwide.

CONCLUSION

As emphasized in the literature, and despite the Rebirthing therapy being practiced and gaining popularity for years, no single published scientific paper directly referring to its physiological aspects, effects, and consequences, was found. Numerous textbooks, guidebooks, and philosophy books have been published about the Rebirthing therapy - all without exception involving solely subjective and prejudiced descriptions and metaphysical explanations about its essence, rationale, physical, mental, emotional, and behavioral effects on healthy and ill participants.

This study looked at the physiological responses during a single Rebirthing session in an objective/scientific manner.

The primary study findings demonstrated that prolonged voluntary hyperventilation (45–50 minutes) causes extremely shallow PETCO_2 levels, high PETO_2 , high RER (relative to resting metabolic requirements), very low VCO_2 , and very high levels of ventilatory equivalents (VE/VO_2 and VE/VCO_2). As specified in the discussion, such conditions might have unpleasant and even risky consequences when lasting for a relatively long period (as during a typical Rebirthing session). However, no significant grievances regarding participants' physical and mental/emotional feelings were reported in this study. In the contrast, at the end of all sessions, patients reported calmness, serenity, and, on occasion, some physical lethargy.

This study could not solve the clear divergence between the observed (acute) physiological responses (mainly severe Hypocapnia) and the subjective participants' pleasant emotional state, and in many cases, spiritually uplifting, at the end of each treatment session. Nevertheless, the authors took the liberty to offer some potential mechanisms by which such conflict could be resolved.

Study limitations: As in most studies, there are some limitations to this study. First, the sample size is relatively small, therefore disapproving conclusive inferences.

Second, we measured both PETO_2 and PETCO_2 as accurate estimates of PaO_2 and PaCO_2 , which are the actual regulators of CBF. Nevertheless, and since PaO_2 and PaCO_2 are not routinely measured (mainly when performed outside of strict medical settings), PETO_2 and PETCO_2 and the changes in both, at rest, and during exercise, closely approximate the changes in both (17,35).

Third, using a face mask during the Rebirthing sessions may lightly interfere with the usual Rebirthing breathing pattern, affecting the accuracy of physiological responses.

Future directions: Rebirthing's mechanism of action may be explored via psychophysiological measurements concurrent with a clinical trial. Advanced neuroimaging techniques, such

as fMRI, may provide a more specific localization of changes in brain activity changes during Rebirthing. To further distinguish between psychological versus direct physiologic effects of Hypocapnia, future studies should examine whether merely prolonged over-breathing, without other aspects of Rebirthing in place, would exert a similar treatment-augmentation impact.

Further, more scientifically oriented studies are needed to determine whether Rebirthing should be a beneficial complementary or alternative treatment for improving well-being and quality of life and common psychiatric disorders.

Using multiple, commonly used outcome measures with well-established psychometric properties and the inclusion of a placebo or wait-list control condition, with random assignment of participants to groups, would significantly enhance the interpretability and validity of findings.

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Conflict of interest: The authors declare no competing interests.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the institutional and/or national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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