Transcutaneous Carbon Dioxide Treatment Affects Heart Rate Variability – A Pilot Study

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Abstract. *Background: The aim of this study was to investigate the effects of carbon-dioxide treatment on heart rate variability (HRV) parameters: mean RR interval (RRI), standard deviation of RR intervals (SDNN), root mean square of successive RR differences (RMSSD); and Porta and Guzik indices, as measures of heart rate asymmetry. Materials and Methods: Twenty patients were enrolled (mean±SD, age=59±7.8 years). Measurements were performed before CO_2 treatment, at the beginning of treatment, at 15 min of treatment, immediately after and 1 h after the treatment. Results: Significant increase in SDNN was found 1 h after the treatment when compared to that before it (p=0.011). There were no significant changes in other parameters. Conclusion: CO_2 treatment can influence the autonomic nervous system identified by SDNN changes. However, larger studies are required to confirm these results.*

The transcutaneous administration of carbon dioxide (CO_2), hereafter referred to as 'CO_2 treatment' has been used for curative purposes for centuries. The first paper investigating the medicinal use of CO_2 was published by Brandi *et al.* in 1932 (1). CO_2 passes freely through membranes and has a well-known vasodilatory effect. Both *in vitro* and *in vivo* studies have demonstrated a rightward shift of the oxygen–haemoglobin dissociation curve after administration of CO_2. Sakai *et al.* described this as an "artificial Bohr effect". This is responsible for the decrease in pH and increase in partial pressure of oxygen in the tissues, through facilitated O_2 release, which were shown *in vivo* (2). The findings of Minamiyama and Yamamoto confirmed these effects using intra-vital video microscopy to demonstrate subcutaneous

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vasodilation after CO_2 administration. In addition, CO_2 was shown to increase blood flow in the observed subcutaneous vessels (3). CO_2 treatment is used to cure several diseases, such as peripheral arterial and venous disorders (*e.g.* claudication, and lower limb ulcer), heart diseases (*e.g.* hypertension, and heart failure) and immunological disorders (*e.g.* Raynaud's syndrome) (4, 5). In short, CO_2 treatment is a non-invasive, highly efficient, low-cost treatment capable of easing the symptoms of arterial and venous diseases possibly due to vasodilatation and reduction of oxidative stress. However, as far as we are aware of, there is no evidence for alteration of the autonomic nervous system after CO_2 treatment.

Heart rate variability (HRV) is controlled by the sympathetic and parasympathetic nervous systems, and is influenced by several other factors (*e.g.* autocrine, paracrine, endocrine, and mechanical stretch). The sinus node is the final summing element in controlling the heart rate. HRV analysis is a non-invasive method providing information on the autonomic regulation of cardiac activity (6, 7). In recent decades, several studies have shown an association between cardiovascular diseases and HRV. HRV analysis is considered a useful tool for predicting the risk of sudden cardiac death after myocardial infarction (8, 9). Numerous studies have investigated HRV among diabetic patients: the most useful finding is that reduced HRV can indicate diabetic neuropathy before clinical symptoms actually appear (10-12). A recent study suggested that HRV can be a potential new tool to predict complications after cardiac surgery (13). In short, HRV assessment is a non-invasive method used to assess the heart's ability to respond and adapt to external or internal stress.

The aim of this study was to investigate the instantaneous effects of a single CO_2 treatment on HRV and heart rate asymmetry (HRA) parameters.

Materials and Methods

The present study was performed at our ISO 9001-accredited Cardiology Rehabilitation Inpatient Unit from July 2017 to January 2018 in Zsigmondy Vilmos SPA Hospital, Harkány. Non-

diabetic, abstinent, hypertensive patients, suffering from coronary heart disease were enrolled. Patients who had previously received CO_2 treatment were excluded. Moreover, patients who had suffered myocardial infarction, stroke or had undergone open heart surgery less than a year before the study were also excluded. Additionally, individuals diagnosed with any kind of cancer or kidney injury were also not enrolled. Informed written consent was obtained from each patient. The study protocol was approved by the Regional Ethics Committee at the University of Pécs, Pécs, Hungary (Permission No. 5919), in accordance with the 2008 Helsinki declaration.

CO_2 gas was administered for 35 minutes in a plastic bag sealed at mid-thoracic level, as previously described by Fabry *et al.* (4). HRV data acquisition was performed using a hand-held, battery-powered device based on a PIC18F46J50 microcontroller (Microchip Technology Inc., Chandler, AZ, USA) and INA333 instrumentation amplifier chip (Texas Instruments, Dallas, TX, USA). The device contains analogue circuitry for a single-channel (three-electrode) electrocardiograph (ECG) amplifier among others. The recordings were transferred to a PC on an SD card for analysis. After extracting the 1-ms temporal resolution ECG signal from the stored data, automatic detection of RR intervals and manual checking was performed by ECGRdet v2.1 software (L. Hejjel, Pécs, Hungary). A 300-s-long artefact-free part of the recording was then selected manually. Particular attention was paid to selecting a portion containing exclusively normal sinus interbeat intervals in order to obtain correct parameters of HRV. Varian v2.2 software (L. Hejjel, Pécs, Hungary) was used to calculate the standard time-domain parameters: mean RR interval (meanRRI), standard deviation of normal-to-normal RR intervals (SDNN) and root mean square of successive RR differences (RMSSD), as well as HRA parameters: Porta index and Guzik index. The hardware and software (ECGRdet and Varian) were developed by L. Hejjel (14-16). Non-invasive blood pressure measurements were made in the middle of each of the five stages of the study. Average respiration frequency was calculated by fast Fourier transformation of the tachogram by Varian v2.2.

During the 3-week-long in-ward rehabilitation, patients underwent a combination of physiotherapies, and received nine CO_2 treatment (35 min per session). In order to follow the changes of HRV parameters, analysis was performed immediately before CO_2 treatment, immediately after the beginning of the CO_2 treatment, 15 min after the beginning of the CO_2 treatment, immediately after the end of the CO_2 treatment, and 1 h after the end of the CO_2 treatment. All the measurements were performed with the patients in supine position 15 min after orthostatic adaptation and from 8am to 10pm in order to avoid positional or diurnal influences, respectively. The patients did not receive any medication besides their regular antihypertensive drugs.

Statistical analysis. Statistical analysis was carried out with StatistiXL package (Broadway-Nedlands, Western Australia) in MS Excel (Microsoft Corp., Redmond, WA, USA). Calculated HRV and HRA measures were assessed by Friedman test and *post-hoc* Wilcoxon test with Holm–Bonferroni correction. The 2-5th stages were compared to the first one. Before correction *p*-values of less than 0.05 were considered statistically significant. Charts were constructed using OriginPro 2017 (OriginLab Corp., Northampton, MA, USA).

Results

Twenty-three patients were enrolled, out of whom three were excluded due to inappropriate ECG signal quality, resulting in 20 patients remaining in the study (age, mean±SD=59±7.8 years). Clinical characteristics of the participants are shown in Table I.

During the entire measurement, there were no significant systolic or diastolic blood pressure changes detected in any patient.

Regarding the meanRRI, there were no significant changes found (Figure 1A). A significant increase in SDNN was detected 1 h after the CO_2 treatment (Friedman test: *p*=0.006, Wilcoxon test: *p*=0.011) (Figure 1B). No significant changes were demonstrated in the RMSSD parameter (Figure 1C).

Regarding the Porta and Guzik indices, no statistically significant differences were found during the monitoring. The effects of CO_2 treatment on Porta and Guzik indices are shown in Figure 2.

The detailed results for HRV parameters measured before, during and after CO_2 treatment are shown in Table II.

The breathing frequency dropped from 0.25 to 0.22 Hz after 15 min of treatment and elevated to 0.27 Hz immediately after CO_2 treatment (Figure 3A). There was no correlation (insignificant *p*-values) between respiration frequency and HRA parameters (Figure 3B).

Discussion

In the present study, the effects of CO_2 treatment on the autonomic nervous system were investigated using HRV and HRA analysis. Our protocol was aimed at minimizing the effects of diurnal changes, body position, vocalization and movements on HRV and HRA parameters. To rule out inter-observer errors, all measurements were performed by the same investigator.

To the best of our knowledge this is the first study investigating the effects of CO_2 treatment on HRV and HRA parameters.

The meanRRI remained stable during the study, reflecting steady state or balanced sympathetic and parasympathetic actions. SDNN showed a mild but continuous increase during the study, which reached statistical significance 1 h after the CO_2 treatment by *post-hoc* test. Since SDNN reveals global variability including both sympathetic and parasympathetic effects (6, 17), some autonomic influence occurs in spite of the steady-state meanRRI. The beat-to-beat variability parameter RMSSD is a measure of vagal actions on heart rate (6, 17). At the beginning of the treatment a slight drop was seen, followed by a monotonous increase not reaching the level of significance. This probably occurred due to the complex cardiovascular actions of CO_2, the high inter-individual variation and the relatively small sample
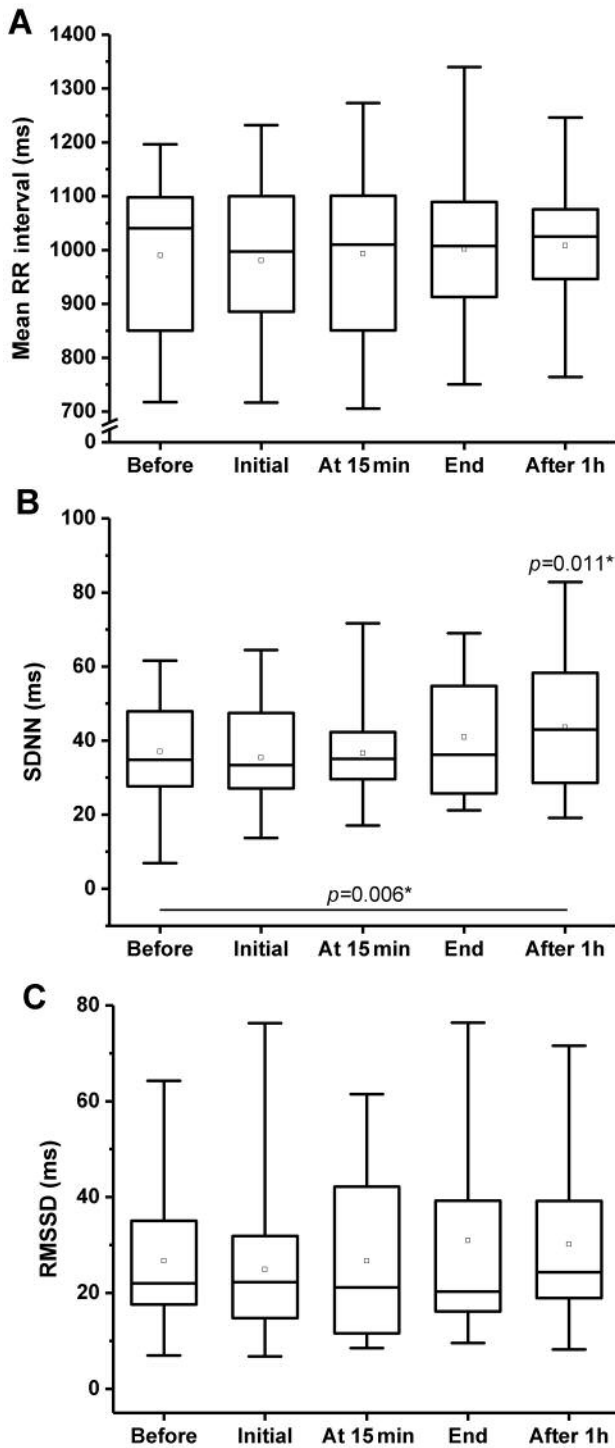
Figure 1. *Time domain measurements before, during (at beginning and at 15 min), and after (immediately and 1 h later) CO_2 treatment. A: Mean time between two consecutive R-waves on the ECG (RR interval). B: Standard deviation of RR intervals (SDNN). C: Root mean square of successive RR interval differences (RMSSD). Boxes: Lower and upper quartiles, horizontal line: median value, whiskers: minimum and maximum values, square: mean value. *Friedman test p-value, **post-hoc Wilcoxon's paired sample test p-values, only significant differences are indicated.*
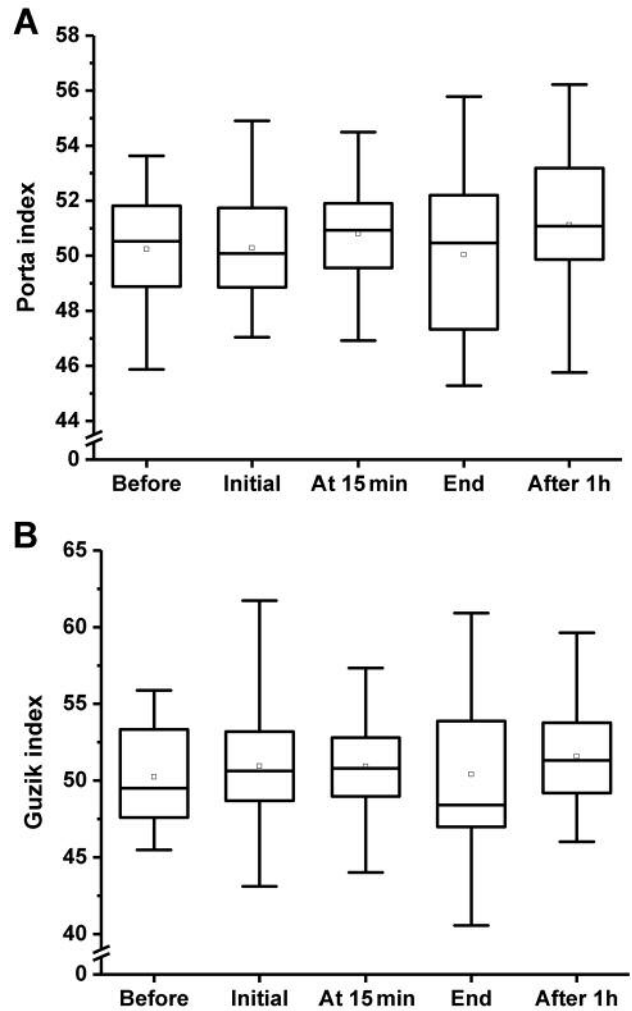

Figure 2. *The effect of CO_2 treatment on Porta (A) and Guzik (B) indices before, during (at the beginning and at 15 min), and after (immediately and 1 h later) CO_2 treatment. Boxes: Lower and upper quartiles, horizontal line: median value, whiskers: minimum and maximum values, square: mean value. Friedman test resulted in p=0.555 for both parameters.*

Table I. *Clinical characteristics of the participants.*

	Patient group (n=20)
Mean age, years (±SD)	59 (±7.8)
Male, n (%)	13 (62)
Diabetes type 2, n (%)	4 (19)
Smoker, n (%)	3 (14)
EF% (mean±SD)	58 (±4.2)
BMI, kg/m² (mean±SD)	27,4 (±5.1)
WBC G/l, (mean±SD)	7.2 (±1.7)
CRP mg/l (mean±SD)	5.3 (±1.9)
HGB mg/l (mean±SD)	136.2 (±11.6)

EF: Left ventricular ejection fraction, BMI: body mass index, WBC: white blood cell count, CRP: C-reactive protein level, HGB: haemoglobin level.
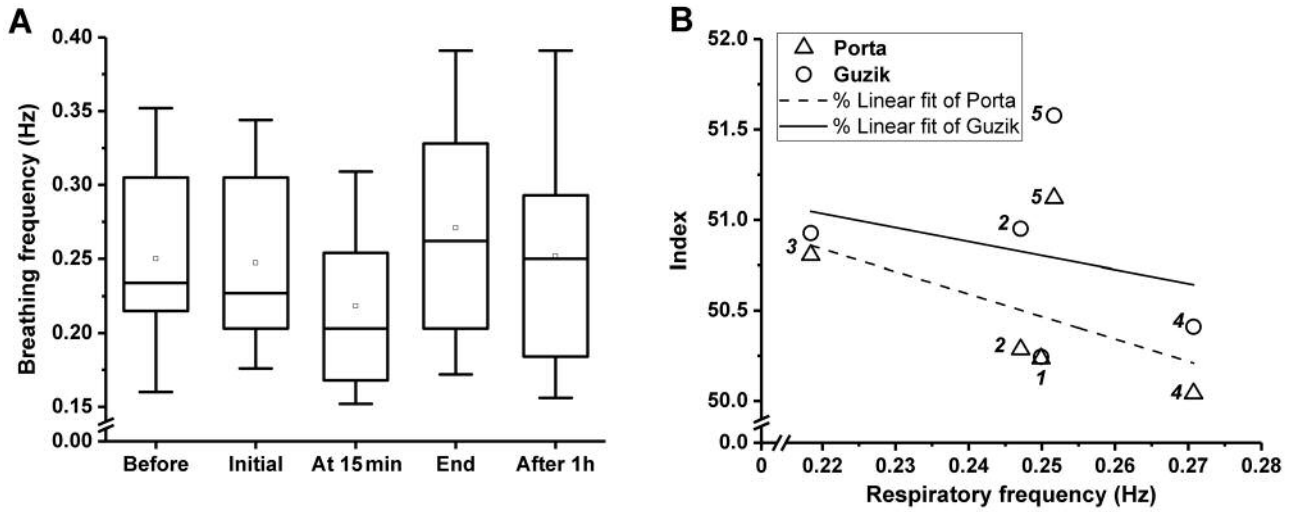
Figure 3. *A: Mean values of breathing frequency derived from spectral analysis of the tachograms. Boxes: Lower and upper quartiles, horizontal line: median value, whiskers: minimum and maximum values, square: mean value. Friedman test resulted in p=0.555. B: Correlation of breathing frequency and heart rate asymmetry parameters with non-significant linear fit. The Arabic numbers in Italics next to data points correspond to the five measurements made. The wide divergence of the Porta and Guzik indices at the beginning of the treatment, immediately after and 1 h after the treatment is remarkable, as well as the elevated indices at the 5th measurement compared to the 1st and 2nd in spite of the similar breathing frequency.*

Table II. *Detailed results of measurements.*

	Timepoint relative to CO_2 treatment				
	Before	At start	After 15 min	After end	1 h After
Mean RRI (ms)	989.8±130.3	985.1±131.87	993.7±138.8	1005.8±136.8	989.8±232.3
SDNN (ms)	35.6±13.1	34.1±13.8	35.9±13.3	40.4±16.1	**42.6±18.5**
RMSSD (ms)	25.1±15.2	23.7±16.2	25.4±16.8	29.7±20.6	28.1±17.8
Porta index	50.3±2.1	50.2±1.6	51.0±1.8	49.9±3	51.3±2.6
Guzik index	50.4±3.5	50.6±4.1	51.7±3.5	50.1±5.1	52.13±3.1

MeanRRI: Mean interval between two consecutive R-waves on the ECG, SDNN: standard deviation of RRI. RMSSD: root mean square of successive RR interval differences. Bold numbers correspond to values significantly different from that before treatment after Holm-Bonferroni correction (p=0.011).

size. Consequently, an increase in both sympathetic and parasympathetic activity can occur during CO_2 treatment, resulting in unchanged heart rate, systolic and diastolic blood pressure. In line with our previous finding, the changes in overall HRV suggest that CO_2 is not simply a vasodilator but is capable of altering the function of the cardiovascular system, possibly by activating endogenous mechanisms leading to vasodilatation (18). Bickel *et al.* found increased sympathetic activity during positive pressure CO_2 pneumoperitoneum by HRV analysis possibly due to hypercarbia and tension of the peritoneum; however, in the present study there was no peritoneal tension (19).

Regarding the Porta and Guzik indices, no statistically significant differences were found during the investigation, once again probably due to the small sample size. However, an increase in both these indices at the end of the treatment and a mild drop immediately after the treatment was observed, although they remained higher even after 1 h compared to the basal level (Table II). According to our previous study, the Porta and Guzik indices are strongly correlated to each other in healthy volunteers even at different inspiration/expiration ratios (16), whereas in the current investigation, their divergence was observed at the beginning of CO_2 treatment, immediately after and 1 h after the

treatment (Figure 3B). The physiopathology of HRA parameters is not well known; therefore, we extracted the average breathing frequency from the tachograms by fast Fourier analysis in order to link the observed HRA changes to breathing rate. Faster breathing corresponds to a shorter expiration period relative to inspiration, theoretically resulting in higher HRA indices, based on the work of Klintworth *et al*. (16). On the contrary, in our study, an opposing change of breathing frequency compared with Porta and Guzik indices was observed (Figure 3B), resulting in a negative regression line. Unfortunately, the correlation was not significant due to the outlier at the final measurement, when in spite of the normalization of the breathing rate, the HRA indices further increased (Figure 3B). This phenomenon again reflects both an instantaneous and a sustained vegetative action of CO_2 treatment, which cannot be explained by our monitored or computed parameters, therefore precise respiration monitoring (via transthoracic impedance or a sensitive chest strap) is recommended in subsequent investigation. This mighty be explained at the second measurement of CO_2 treatment by the Bohr effect, when enough CO_2 accumulates in the body to facilitate O_2 release from haemoglobin (2, 3), resulting in slower breathing as a response to higher oxygen tension in the chemoreceptor area. After finishing the treatment, the rapid diffusion and exhalation of CO_2 causes the Bohr effect to cease, which stimulates the breathing centre, resulting in a transient higher breathing immediately after the treatment (Figure 3A).

The relatively small number of participants may be the reason for the insignificant differences in RMSSD and HRA parameters.

In conclusion, the reported study protocol is adequate for investigating the effects of CO_2 treatment on HRV and HRA parameters. According to the results of this pilot study, CO_2 treatment can influence the autonomic nervous system. However, larger studies with respiration monitoring are required to confirm these results and to discover the mechanism.

Conflict of Interest

The Authors declare that they have no conflict of interest in regard to this study.

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Ethical Approval

All procedures performed in the study were in accordance with the ethical standards of the Regional Research Ethics Committee and with the 1964 Helsinki declaration and its later amendments. The study protocol was approved by the Regional Ethics Committee of University of Pécs, Pécs, Hungary (Permission No.: 5919.)

Acknowledgements

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