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 Güzik 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 CO₂ 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: CO₂ 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 (CO₂), hereafter referred to as ‘CO₂ treatment’ has been used for curative purposes for centuries. The first paper investigating the medicinal use of CO₂ was published by Brandi et al. in 1932 (1). CO₂ 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 CO₂. 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 O₂ release, which were shown in vivo (2). The findings of Minamiyama and Yamamoto confirmed these effects using intra-vital video microscopy to demonstrate subcutaneous vasodilation after CO₂ administration. In addition, CO₂ was shown to increase blood flow in the observed subcutaneous vessels (3). CO₂ 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, CO₂ 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 CO₂ 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 CO₂ 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-
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 CO₂ 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 CO₂ treatment on Porta and Guzik indices are shown in Figure 2.

The detailed results for HRV parameters measured before, during and after CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂, the high inter-individual variation and the relatively small sample.
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.
size. Consequently, an increase in both sympathetic and parasympathetic activity can occur during CO₂ treatment, resulting in unchanged heart rate, systolic and diastolic blood pressure. In line with our previous finding, the changes in overall HRV suggest that CO₂ 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 CO₂ 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 CO₂ treatment, immediately after and 1 h after the

Table II. Detailed results of measurements.

| Timepoint relative to CO₂ 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        | 52.1±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 ($p=0.011$).
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 CO₂, treatment can influence the autonomic nervous system. According to the results of this pilot study, CO₂ 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.)

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