Project thesis - MED5090

Heart rate variability: exploring the influence of increased carbon dioxide levels in the breathing air

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Abbreviations

CBF - Cerebral blood flow

ECG – Electrocardiogram

$ETCO_2$ - End-tidal carbon dioxide

HR - Heart rate

HRV - Heart rate variability

IAP - Intraabdominal pressure

IPPV – Intermittent positive pressure ventilation

ITP - Intrathoracic pressure

LBNP - Lower body negative pressure chamber

MAP - Mean arterial pressure

NIPPV – Non-invasive intermittent positive pressure ventilation

NTS – Nucleus of the solitary tract

$PaCO_2$ - Partial pressure of arterial carbon dioxide

PEEP - Positive end-expiratory pressure

PSD - Power spectral density analysis

RSA - Respiratory sinus arrhythmia

TV - Tidal volume

Introduction (“Kappe”)

Norwegian summary

Sirkulasjon og respirasjon er i et viktig samspill. Dette samspillet er avgjørende for kontrollen av det kardiovaskulære systemet. De sirkulatoriske og respiratoriske variablene inneholder mange svingninger. Eksempler på slike svingninger er blant annet hjerteslag, blodtrykk og respirasjon. Disse svingningene kan være en del av viktige kontrollmekanismer kroppen bruker for å håndtere plutselige stressende situasjoner (både fysiske og mentale) som den møter på. En av de mest studerte svingningene er hjertefrekvensvariabilitet (HRV) og kan forstås som variasjonen i tid mellom to påfølgende hjerteslag. Generelt vil en høy HRV anses som en indikator for et sunt hjerte og vil være redusert ved en rekke forskjellige sykdommer. I tillegg til å kunne si noe om hjertets tilstand, så kan HRV også brukes for å forstå det autonome nervesystemets tilstand, som er delansvarlig for å regulere hjerteaktiviteten.

En viktig del av HRV er respiratorisk sinus arytmi (RSA). RSA er en forbindelse mellom det sirkulatoriske og respiratoriske systemet og er et viktig fysiologisk fenomen. RSA er når hjertefrekvensen øker ved inspirasjon og minker ved ekspirasjon. Den utgjør 20-60 % av korttids HRV i hvile hos mennesker. Tap av RSA er assosiert med flere sykdommer og økt mortalitet, men denne sammenhengens årsaksmekanismer er derimot ukjent. Flere faktorer og mekanismer bidrar til å påvirke RSA, som blant annet pustemønster, søvn og fysisk aktivitet. En annen viktig faktor som påvirker RSA og som denne oppgaven skal fokusere på, er arteriell CO_2, som gjennom stimulering av kjemoreseptorer vil utover ventilasjonsforandringer, føre til endring av nervus vagus sin aktivitet på hjertet og dermed RSA.

I 2018/2019 ble det utført en eksperimentell studie av vår forskergruppe. Vi analyserte en del av dette datasettet i 2022 for denne prosjektoppgaven. Vi undersøkte hvordan RSA og HRV ble påvirket når forsøkspersoner pustet inn luft med tilblandet CO_2, og ønsket å kvantifisere effekten CO_2 hadde på RSA og HRV. I denne oppgaven har vi også satt dette inn i den gjeldende litteraturen på området. Siden RSA og HRV er redusert ved flere forskjellige sykdommer, så kan det å utforske hvilke faktorer og interaksjoner som bidrar til å øke RSA og HRV være nyttefull for å behandle og forebygge kardiovaskulære- og respiratoriske sykdommer.

Abstract

Background

There is a vast variety of oscillations within the cardiorespiratory system. One of the most studied oscillations is heart rate variability (HRV) in the high-frequency interval (0.15 – 0.4 Hz). Respiratory sinus arrhythmia (RSA) is a physiological phenomenon we can observe in several species and is a significant contributor to HRV. RSA, which is influenced by several factors, including ventilation pattern, is more prominent in young people and diminishes with age, several diseases, and mechanical ventilation. In this study, we recruited healthy volunteers and investigated the impact of adding 5 % CO_2 to the breathing air on high-frequency HRV. We also wanted to quantify this effect on high-frequency HRV.

Methods

Thirteen healthy subjects of both genders (5 males) were recruited for the original study after written informed consent. Three-lead electrocardiogram (ECG), Heart rate (HR), stroke volume (SV), mean arterial pressure (MAP), plethysmographic finger arterial blood pressure (ABP), end-tidal CO_2 ($ETCO_2$), and respiratory airflow were recorded during spontaneous breathing, mask breathing, and CO_2 breathing. The RR time was the distance between two R waves of a QRS signal on the ECG. The mean value and variability for every cardiovascular and respiratory variable for every person were calculated using SIGVIEW. As for cardiovascular variability for the high-frequency interval, this was calculated using fast Fourier transform spectral analysis. We then calculated medians and 95 % confidence intervals using the Hodges-Lehmann estimate, while also testing the difference between the conditions using the Wilcoxon signed-rank test for paired samples. We also assessed the effects of HR, tidal volume (TV), and $ETCO_2$ on HRV by linear mixed model regression analysis, and statistical significance was set to $P < 0.05$.

Results

Breathing the CO_2 mixed air influenced TV and cardiovascular variability markedly. Compared to spontaneous breathing, TV and HRV tripled, while SV variability was doubled. As for MAP variability, a tenfold increase compared to spontaneous breathing was observed. HR showed minimal changes between the conditions. $ETCO_2$, compared to spontaneous breathing,

decreased slightly during mask breathing, and increased greatly during CO_2 breathing as expected. Linear mixed models regression analysis showed that the level of tidal volume and $ETCO_2$ (both $p < 0.01$) were both positively associated with HRV, while HR ($p = 0.62$) was not.

Conclusion

Our findings indicate that elevated levels of both $ETCO_2$ and tidal volumes correlated with an augmentation in high-frequency HRV. A rise in $ETCO_2$ was observed to triple high-frequency HRV. An unexpected finding was the tenfold increase in MAP variability during CO_2 breathing. We are cautious in drawing conclusions regarding the impact of $ETCO_2$ within physiological limits.

Supervisor Statement

The main supervisors (Maja Elstad and Lars Walløe) confirm that Yasin and Atiq have performed the analysis, literature search and written the project thesis. The total workload is estimated to 12 weeks for each of the students. Both students have contributed equally to the project thesis.

Our contribution to the extended abstract and work with the project thesis

This project thesis is written by both Atiq Mohammad Ayoubi and Yasin Ali Hassan. We enrolled in the medical research program at the University of Oslo in January 2022 after finishing our third year in medicine. We had just stepped foot in the world of research, and our preliminary knowledge of this realm was very little. The main experimental research that we were going to be working on for the medical research program was about the effect of inspiratory resistance breathing on cardiac output and cerebral blood flow during hypovolemia. Before embarking on this journey, our supervisor, Maja Elstad wanted us to become familiar with the processes involved in conducting research. We were presented with an experimental

study done by our research group that took place in 2019 that investigated the effects of hypercapnia on cerebral blood flow (CBF).

The first program that we started with was GraphPad, a program used for making graphs and presenting data. After getting familiarized with the program and making several graphs for different variables, we moved on to analyzing the subject data from the study using a data analysis program called SIGVIEW. We both did the same analysis on SIGVIEW separately so that we both got used to the analysis program and so that we could compare our findings afterward. For the statistical part, we used another analysis program called StatXact. Just like SIGVIEW, we both performed the same analyses for StatXact.

The last analysis we did was a linear mixed models regression analysis using a program called JMP Pro. This part of the data analysis was taught by Professor Signe Søvik. She showed us how to use the program, make graphs, and perform regression analyses. After collecting all the data we needed from the different analysis programs, we started making graphs and tables out of the data, which you can find at the end of this document. Yasin made the figures while, Atiq made the tables. After finishing all the analyses, we worked on writing an extended abstract out of the work we had done, which can be found on page 38 of this document. The writing of the extended abstract was thanks to fruitful discussions and help from our supervisor Maja Elstad, co-supervisors Lars Walløe and Maria Skytioti, and Professor Signe Søvik. We submitted the extended abstract and held an oral presentation at the 12th conference of the European study group on cardiovascular oscillation (ESGCO) in Slovakia in October 2022.

After writing and presenting the extended abstract, we moved on to writing the introduction “Kappe”, where also Dr. Julia Gundersen helped us in addition to those mentioned above. For the writing of the extended abstract and the introduction “Kappe”, we used a shared Google Docs document so that we could track each other's writing. In the collaborative creation of the introduction “Kappe”, Atiq Mohammad Ayoubi was responsible for writing the Norwegian summary, abstract, outlining our contribution and work, detailing the subject and ethical considerations, and explaining the experimental protocol, and how we conducted the measurements. Yasin Ali Hassan was responsible for addressing the background, discussing the measurement of heart rate variability, writing the discussion section, and formulating the conclusion. For the writing of the extended abstract, Atiq was responsible for writing materials and methods and results. Yasin took charge of the abstract, introduction, and discussion. During

all parts of the project thesis, we were constantly consulting each other and giving each other suggestions, thereby influencing each other's writing. The total duration of our work was 24 weeks: 2 weeks each for the data analysis, 4 weeks each for writing the extended abstract and preparing for the ESCGO conference, 2 weeks each for reading literature on the field, and 4 weeks each for writing this project thesis.

Background

To better understand HRV and RSA, and the intricate interplay between the cardiovascular and respiratory system, we first need to discuss how these systems work and are regulated. In this section, we will therefore explore the physiology and regulation of heart rate and blood pressure, and also write about the respiratory system and how it is influenced by CO_2. We will then discuss HRV and RSA, addressing the most relevant mechanisms contributing to the regulation of RSA, as it can be influenced by many factors.

A. Cardiovascular system

Heart rate is provided by specialized cardiac muscle cells. The main location of these cells is in the right atrium. These cells initiate an action potential, leading to an electrical impulse traversing the heart's conduction system and inducing myocardial contraction. Unlike atrial and ventricular cells, pacemaker cells in the sinus node lack a resting phase (figure 1). Instead, they exhibit pacemaker potential, automatically initiating depolarization following the conclusion of an action potential (1).

The sinoatrial node is innervated by both sympathetic and parasympathetic branches of the autonomic nervous system, which regulate the heart rate from the baseline of 100 beats per minute (2). During stressful situations, sympathetic neurotransmitters, including adrenaline and noradrenalin, specifically bind to beta-adrenergic receptors on the cells of the sinoatrial node and cardiomyocytes. This binding results in increased heart rate and stronger contractions. During calm situations, the neurotransmitter acetylcholine will be released and bind to muscarinic receptors on the surface of heart muscle cells. Activation of these receptors results in a reduced heart rate.

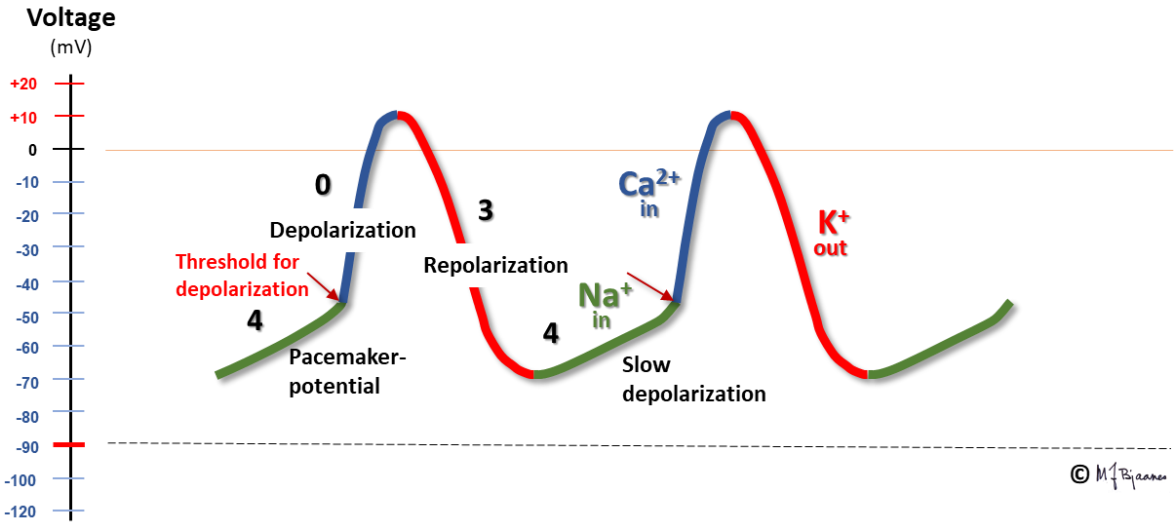

Figure 1. A typical action potential in the sinoatrial node. The action potential is divided into four phases. It begins with the pacemaker cell gradually depolarizing in phase 4, enabled by the influx of sodium ions (Na+). This results in a gradual rise in membrane potential. The subsequent influx of calcium ions (Ca2+) stimulates a rapid depolarization, marking the initiation of the action potential in phase 0.

In phase 3, potassium channels are fully open, facilitating an efflux of potassium ions (K+) and full repolarization. In phase 4, the cell returns to its resting membrane potential, gradually beginning the depolarization process and initiating the subsequent action potential. The figure was made by Michael Bjaanes, and it is reused with his permission.

Arterial baroreceptors also influence heart rate. Baroreceptors are stretch-sensitive free nerve endings found mainly in the arch of the aorta (arcus aortae) and the internal carotid arteries, known as the carotid sinuses. These receptors send action potentials at a specific frequency to the cardiovascular center in the medulla oblongata. Afferent signals from the carotid sinus are conveyed through afferent fibers of cranial nerve IX, the glossopharyngeal nerve. At the same time, afferent information from the aortic arch is communicated through the vagus nerve. If these nerve endings are stretched due to increased pressure, they transform the stretch of the vessel into action potential and increase their firing, leading to a reduction in heart rate in less than a second. Conversely, reduced stretch on these nerve endings will result in decreased baroreceptor firing and increased in heart rate and cardiac output (figure 2) (3).

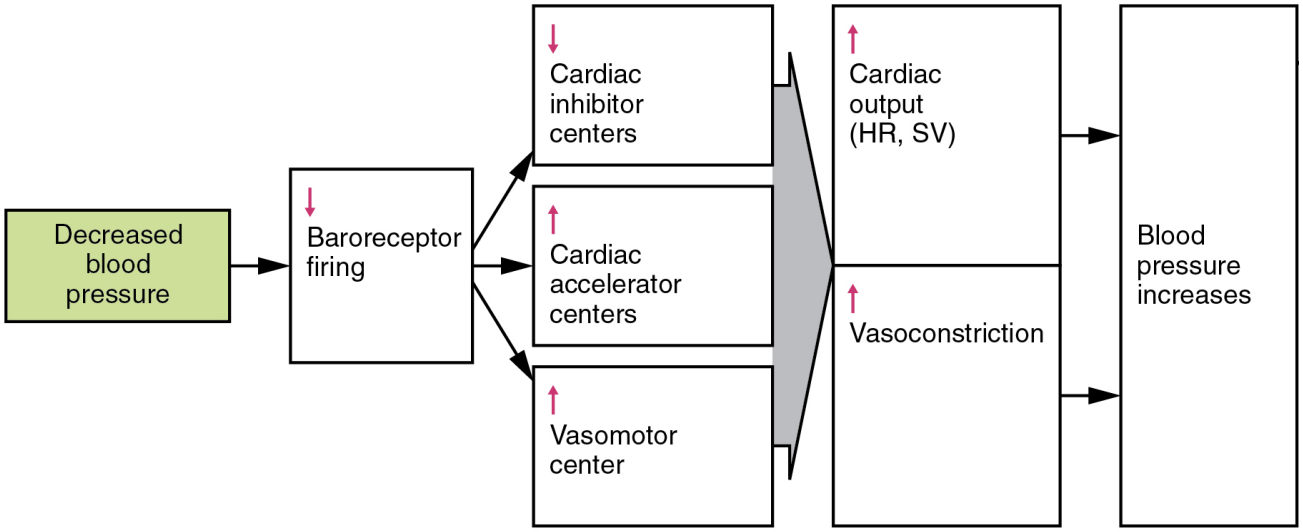

Figure 2. A drop in arterial pressure causes a reduction in baroreceptor activity, this trigger different centers in the cardiovascular center in the medulla oblongata. The net result is increased cardiac output and vasoconstriction (4).

Volume receptors also influence heart rate. They are located in the large veins and the atria of the heart. These receptors detect changes in blood volume. If the atria of the heart are stretched, natriuretic peptides such as atrial natriuretic peptide (ANP) are released, prompting the kidneys to excrete more urine. An increase in blood volume will reduce heart rate, while a decrease in blood volume will increase heart rate. This humoral reflex from volume receptors is slower compared to baroreceptors, and the response occurs after 20-30 seconds following stimulation of the volume receptor.

The cardiovascular center in the medulla oblongata is a crucial center for coordinating the sympathetic and parasympathetic nervous systems. This center receives afferent information from both sympathetic and parasympathetic nerve endings, acting as sensors in the circulatory system. In this area, the nucleus of the solitary tract (NTS) functions as the central location for the initial synapse of cardiovascular and baroreceptor afferents, playing a crucial role in integrating inputs. The NTS integrates and transmits cardiovascular and baroreceptor afferent information through a polysynaptic pathway to other significant medullary centers. This process regulates parasympathetic and sympathetic pathways governing the heart and blood vessels (3).

B. Respiratory system

Respiration is the process of exchanging oxygen and carbon dioxide between the body and the atmosphere. It involves inhaling oxygen into the lungs, where it is absorbed into the blood in the capillaries of the lungs and then distributed to all cells in the body. In the opposite direction, blood carries carbon dioxide from the body's cells through the bloodstream to the lungs, where the gas is exhaled. Respiration is a dynamic alternation of inspiration and expiration produced by the contraction and relaxation of respiratory muscles. The respiratory system is closely regulated to ensure a constant partial pressure of oxygen and carbon dioxide in the arterial blood (5). The control of the respiratory center in the brain is a collaborative effort between the pons and medulla (6). Together, these neural control centers manage the intricate regulation of both inspiration and expiration. Specifically, the dorsal medulla takes charge of inhalation, while the ventral medulla oversees exhalation.

The signaling strength originating from the dorsal medulla holds significant control over the breathing process. An increased frequency of impulses leads to more robust muscle contractions and deeper breaths, whereas a decreased frequency results in a passive expiration (7). The dorsal medulla communicates with the ventral medulla by integrating input from central and peripheral receptors. This integration occurs prior to transmitting information to respiratory muscles, effectively generating the rhythmic pattern of breathing (7).

CO_2 is a potent stimulant for respiration. Central chemoreceptors are sensors located in the brainstem responsible for sensing pH, O_2, and CO_2 concentrations in the brain and the cerebrospinal fluid (8). As the partial pressure of carbon dioxide in arterial blood elevates, ventilation increases nearly linearly. Carbon dioxide makes the environment in the brain more acidic; this triggers the respiratory center to initiate inspiration and contract inspiratory muscles such as the diaphragm and the intercostal muscles (9). Consequently, there is an elevation in both the rate and depth of respiration, facilitating the expulsion of more CO_2 and thereby decreasing CO_2 levels and hydrogen ions in the blood. Conversely, diminished levels of CO_2 in the blood result in reduced levels of hydrogen ions in the brain, ultimately leading to a decline in the rate and depth of ventilation, causing a slowdown in breathing (9).

Peripheral chemoreceptors on the other hand are located in strategic areas of the body, specifically in the vascular wall of the aortic arch and the bifurcation of the common carotid artery (the carotid sinus). The carotid bodies are also exceptionally well-perfused structures and able to monitor the partial pressures of oxygen and carbon dioxide in the arterial blood and respond to acidosis, hypercapnia, or hypoxia (5). The sensory nerve connected to the carotid body exhibits a hyperbolic increase in its firing rate when the partial pressure of oxygen decreases. In response to hypercapnia, the carotid body increases its activity as the partial pressure of carbon dioxide is raised. The signals from the peripheral chemoreceptors are transmitted to the respiratory center in the brainstem, influencing the depth and frequency of our breaths.

Voluntary respiration, also known as conscious or controlled respiration, refers to the deliberate and intentional regulation of the respiratory process by an individual. In contrast to automatic or involuntary breathing, which is regulated by the brainstem to ensure the body's basic oxygen and CO_2 exchange needs, voluntary breathing involves conscious effort and awareness (12).

In voluntary breathing, an individual can consciously modify the rate, depth, and rhythm of their breaths. This can be done for various purposes, such as relaxation, meditation, or specific activities like singing or playing wind instruments. The primary muscles involved in voluntary breathing are the diaphragm and the intercostal muscles, which work together to expand and contract the chest cavity, facilitating air movement in and out of the lungs (10).

Mechanical ventilation is an additional way breathing can occur. Instead of creating a negative ITP as would happen when breathing normally, air can also be “pushed” into the lungs by intermittent positive pressure ventilation noninvasively (NIPPV) or invasively (IPPV), thereby attenuating the negative ITP or even creating a positive ITP instead during inspiration. Positive pressure ventilation increases the delivery of oxygenated air to the alveoli and is indicated for use in many situations such as during hypoxemic respiratory failure, to prevent atelectasis, or to maintain an open airway (11). (Extrinsic) Positive end-expiratory pressure (PEEP) is the therapeutic parameter set in the ventilator during mechanical ventilation and is the positive pressure that will remain in the airways at the end of exhalation. PEEP is not to be confused with auto-PEEP, a complication of mechanical ventilation with air trapping (12).

C. Heart rate variability and respiratory sinus arrhythmia

HRV refers to the variation in duration between consecutive heartbeats, reflecting the dynamic interplay between the sympathetic and parasympathetic branches of the autonomic nervous system. The HRV that occurs in the high-frequency interval (0.15 to 0.4 Hertz) is known as high-frequency HRV. Oscillations in the heart rate in the high-frequency interval often align with our breathing pattern and give rise to RSA. RSA is HRV in synchrony with respiration, by which the R-R interval on an ECG is shortened during inspiration and prolonged during expiration. RSA is one of many cardiorespiratory interactions and is also the most studied aspect of HRV. Cardiorespiratory interactions are the dynamic interplay between the cardiovascular and respiratory systems.

RSA, a significant contributor to HRV, exhibits a decline with age. Various factors contribute to this reduction, including age-related changes in the autonomic nervous system. Additionally, alterations in respiratory function, shifts in cardiovascular dynamics, the prevalence of age-related health conditions, and lifestyle factors may collectively influence the observed decrease in RSA over time (13).

D. Regulation of respiratory sinus arrhythmia

RSA is influenced by several factors, including central neural networks, feedback from peripheral reflex mechanisms, and intrinsic mechanisms (14, 15). In this section, we look further at the first two of these regulatory mechanisms, as the last one has little physiological significance.

I. Central regulation

Central regulation of RSA involves intricate connections between the respiratory and cardiovascular centers in the brain. These connections include the medulla and pons, which play key roles in controlling both breathing and heart rate. During the respiratory cycle, the central pattern generators in the medulla generate rhythmic signals for breathing. The

interactions between the respiratory and cardiovascular centers lead to the modulation of vagal outflow, which lead to rhythmic fluctuations in heart rate, with the heart rate increasing during inspiration (16).

II. Peripheral reflex mechanisms

During spontaneous inspiration, the chest cavity expands, creating a negative intrathoracic pressure (ITP) and a positive intraabdominal pressure (IAP). Stretch receptors in the lungs, are mechanoreceptors located in the smooth muscles of the bronchi, are increasingly activated with rising lung volume (17). The impact of these stretch receptors was investigated by contrasting RSA levels in individuals who underwent lung denervation as part of a double-lung transplant with those in normal subjects. The results revealed that normal subjects with intact stretch receptors exhibited a 50% higher RSA amplitude compared to the patient group (18). The impact of central respiratory feedforward mechanisms was demonstrated in a study by comparing RSA in healthy individuals during spontaneous breathing, and when using NIPPV. The study revealed that individuals experienced a decrease in RSA, up to 60%, when employing (19).

The negative ITP and positive IAP enhances venous blood flow back to the right atrium (20). The increased blood filling of the heart results in elevated stroke volume through the Frank-Starling mechanism. The Frank-Starling mechanism implies that as the volume of blood returning to the heart (venous return) increases, the walls of the heart chambers stretch, causing the cardiac muscle fibers to elongate. This stretching results in a more forceful contraction during the subsequent systole, leading to an increased ejection of blood into the arteries (21). This adaptive mechanism enables the heart to effectively synchronize with varying venous return and ventricular filling volume on a beat-to-beat basis (22). The subsequent change in stroke volume and blood pressure is detected by arterial baroreceptors, which decrease heart rate through parasympathetic activation. During expiration, the opposite occurs, with decreased venous return to the heart and consequently less blood filling of the heart. This, in turn, affects baroreceptors and the cardiovascular center (23).

Research questions

In this study, we aimed to investigate how short-term high-frequency (0.15 - 0.4 Hz) HRV and RSA are affected in young, healthy individuals when breathing air mixed with 5% CO_2. The data is taken from a preliminary study that investigated whether the supply of CO_2 in inhaled air could increase blood flow to the brain. This part of the study that we have performed examines how the circulatory system reacts to increased CO_2 levels. We were aware that CO_2 affects ventilation, and that ventilation affects RSA, but we also wanted to investigate how CO_2 alone affects RSA. Studying the impact of elevated CO_2 on HRV and RSA could help us better understand the physiological response of breathing CO_2 mixed air on the cardiovascular and respiratory system. This could also have some clinical implications as both HRV and RSA are reduced in several diseases.

The questions we aimed to answer were:

i) How would an increase in $ETCO_2$ impact high-frequency HRV and RSA?

ii) How would respiratory variables, cardiovascular parameters, and cardiovascular variability be affected during spontaneous breathing with an open-ended face mask, positive end-expiratory pressure (PEEP) set at 2 cmH_2O using a ventilator, and breathing through a ventilator with PEEP of 2 cmH_2O along with a 5% CO_2 blend in the air?

Materials and methods

A. Subjects and ethical considerations

As described earlier, the data we have used for this project is derived from an experimental study done by our research group. They recruited 13 subjects of both genders (5 males, 23 years [range 19-28], body mass index 23 kg/m2 [19-26]). Including both genders was important for ensuring generalizability and avoiding gender biases (25). All the subjects included were young and healthy, and all were non-smokers. None of the subjects were taking any medications except for contraceptive pills. Since psychological stress can affect the control systems, each participant was familiarized with and examined individually by a standardized protocol.

After written and oral information was provided, all subjects gave written informed consent. The subjects were also informed that they could withdraw at any time and halt the experiment at any moment without having to provide any reason. The study was approved by the regional ethical committee (REK ref. nr. 12589) and was in line with the Helsinki Declaration. All identifiable information would be handled with relevant legislation and was anonymized. To avoid psychological stress as much as possible, all measurements were non-invasively recorded and did not imply any pain or risks. The protocol moments that could be viewed as stressful were during mask breathing and CO_2 breathing. A physician was always present during the experiments.

B. Experimental protocol

The part of the experimental protocol that we analyzed and we present in this project thesis lasted 15 minutes (figure 3). The LBNP chamber was off during this time. The protocol started with the subject supine breathing spontaneously for 5 minutes through an open-ended face mask. After breathing spontaneously, the face mask was attached to a breathing circuit on a ventilator (Maquet Servo-i). The ventilator settings were adjusted at minimum (2 cmH_2O) positive end-expiratory pressure (PEEP). Following 5 minutes of breathing medical air provided by mechanical ventilation, the circuit was introduced to 5 % CO_2 mixed in the air for the next 5 minutes. The protocol would halt immediately if the subject wished to end the experiment.

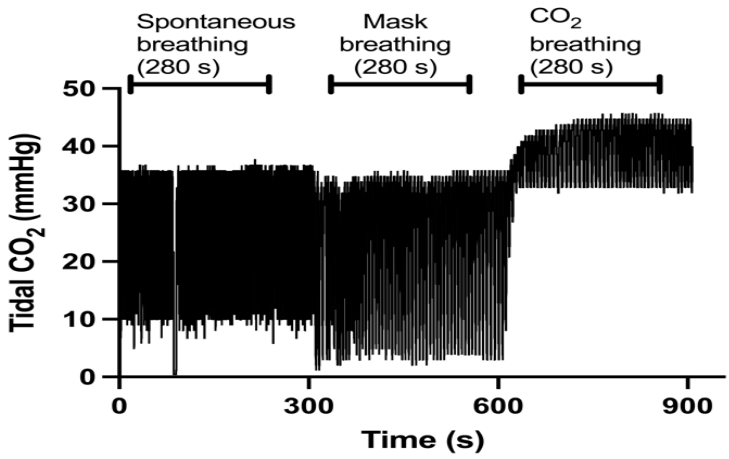

Figure 3. Exprimental protocol displaying subjects´ breathing conditions. The protocol started with 5 min (~ 300 seconds) of spontaneous breathing, then 5 min of mask breathing and ended with 5 min of CO_2 breathing.

C. Measurements

By measuring the distance between two R peaks (RR interval) from a three-lead electrocardiogram (ECG) (SD-100, Vingmed, Horten), HR was calculated beat-by-beat as 60/RR interval. The ECG data was sampled at 300 Hz and not at 100 Hz, as sampling ECG at 100 Hz may be too low for HRV analysis, especially for frequency domain indices (27). Sampling the ECG data at 300 Hz was important as many studies argue that a low sampling rate can result in problems with identifying QRS complexes and inaccurate RR intervals, therefore distorting HRV analysis (24)

Finger arterial p ressure (AP) was obtained through a Finometer (Finometer, Finapres Medical System, the Netherlands). Pulse rate and stroke volume (SV) were also provided by the finometer by the Modelflow algorithm (25). The Modelflow algorithm calculates aortic flow and quantifies SV by integration of a three-element model that includes aortic impedance, arterial compliance, and estimated peripheral resistance. Both aortic impedance and arterial compliance are estimated from aortic pressure-area relationships and also adjusted to individual participant demographics (26). Beat-by-beat MAP was calculated from the blood pressure wave.

A flow head (Hamilton Medical) connected to a custom-made differential pressure transducer was used to measure respiratory airflow. For the recording of $ETCO_2$, a tidal capnography (Cap10, Medlab GmbH, Germany) was placed near the nares. Several studies have shown that measurement of $ETCO_2$ correlates well with partial pressure of arterial carbon dioxide ($PaCO_2$) in both spontaneous breathing and mechanically ventilated patients, even during episodes of severe hypocapnia (27). Besides ECG data that was sampled at 300 Hz, all other data was sampled at 100 Hz into a recording computer (Regist, Morten Eriksen, Oslo, Norway).

D. Measurement of heart rate variability

In 1996, the European Society of Cardiology and the North American Society of Pacing and Electrophysiology established a task force to create standards for the measurement, physiological interpretation, and clinical application of HRV. The resulting guidelines aimed to standardize the terminology and methodology used in HRV measurement. These guidelines encompass various approaches, including linear measures like time domain and frequency domain measures, as well as non-linear measures such as the Poincaré plot (31). We used linear measures within the frequency domain for our calculations, and we will elaborate on this particular methodology.

I. Frequency domain methods

The assessment of HRV can be conducted by using frequency domain method. The frequency domain method converts signal from the time domain to the frequency domain (Hertz) by using fast Fourier transformation (FFT) - a mathematical equation developed by the French mathematician Joseph Fourier. Frequency domain displays how much of the signal exists within a given frequency band concerning a range of frequencies. After the transformation one can perform power spectral density analysis (PSD). PSD is the measure of signal´s power content over different frequencies. PSD analysis is typically performed using the FFT. FFT makes it possible to detect and quantify the amount of cyclic variation present at different frequencies (28). This information is often presented graphically by plotting the degree of variation in a recording on the vertical y-axis against the corresponding frequency on the horizontal x-axis. (figure 4). Measuring the area under the curve at different frequencies, provides a numerical

measure of the amount of high and low-frequency cyclical variability present in the recording (28).

Frequency domain analysis involves calculating variability within periods of different frequencies. The shortest periods (0.15 – 0.4 Hz) is referred to as high-frequency (HF) recording (29). These periods last 2.5 – 7 seconds. Only the parasympathetic nervous system has the capacity to influence heart rate within such short time frames (29). The slightly longer periods (0.04 – 0.15 Hz) are termed low-frequency (LF) recording, and these usually last 7 – 25 seconds. Both the parasympathetic and sympathetic nervous systems have the ability to impact heart rate during low- frequency recording (0.04 – 0.15 Hz). The ECG sampling used for the analysis must undergo extensive editing and review for ectopic heartbeats and other artifacts before conducting frequency domain analysis (28).

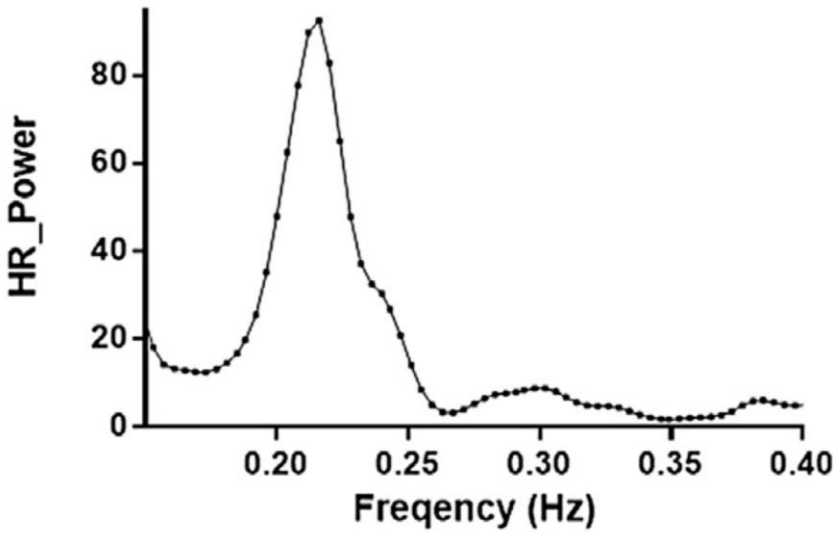

Figure 4: Typical recording of heart rate and power spectrum of high-frequency interval. RSA is estimated as the area under the curve (0.15–0.4 Hz) (19).

E. Analyses

Before we started the analyses, we visually inspected all signals for noise. Two subjects had to be excluded from the analysis because of noise in the ECG recording during CO_2 breathing. Using SIGVIEW (SIGVIEW, version 5.3.2, http://www.sigview.com), a versatile signal analysis software, we calculated each participant's mean value and variability of every respiratory and cardiovascular variable. When it comes to mean tidal volume, we calculated it by randomly choosing ten breaths for each condition and then calculating the flow integral of these breaths. $ETCO_2$ was attained from calculating the maximum CO_2 values of the expiratory phase. As for the variability of the cardiovascular variables, they were calculated using fast Fourier transform spectral analysis. This analysis is one of the two most commonly used methods for power spectral analysis of HRV, the other method being autoregressive models (30). The Fast Fourier transform program is straightforward, readily available, and shows good reproducibility but requires equal distances between the RR intervals, something that requires artificial interpolation. The interpolation can, unfortunately, introduce potential biases (30).

We calculated power spectral density integrals for the high-frequency interval 0.15-0.4 Hz for all conditions in 9 out of 11 subjects. The frequency interval did not fully cover the respiratory frequency for these two remaining subjects, so we had to laterally adjust the frequency interval later to cover their respiratory frequency as well (figure 5). The respiratory frequency was attained by identifying the peak in the frequency power spectra of respiratory activity within the range of 0.05 - 1.0 Hz. The data from each of the three conditions were analyzed in 280 s time periods.

We used non-parametric methods for calculating medians and testing differences between the conditions. Hodges-Lehmann´s estimate was used to calculate the medians and their 95 % confidence intervals. As for testing the differences between the conditions, we used the Wilcoxon matched-pairs signed-rank test against a two-sided alternative, a test that assumes independent observations and symmetrical distribution (31) (StatXact, Cytel Studio 10, Cytel Inc., Cambridge, MA, USA).

After calculating the medians and comparing them between the conditions, we used mixed model linear regression analysis in JMP Pro 16 (jmp.com). The mixed model analysis includes both fixed effects and random effects, and has the advantage of accommodating unequal

numbers of observations across subjects, and can also handle the correlation between repeated observations within a given subject (32). High-frequency HRV was selected as the dependent variable, while $ETCO_2$, tidal volume, and HR were selected as independent variables. The independent variables were chosen after entering all explanatory variables and systematically removing them one by one, starting with the least significant. Statistical significance was set to $p < 0.05$ (figure 5).

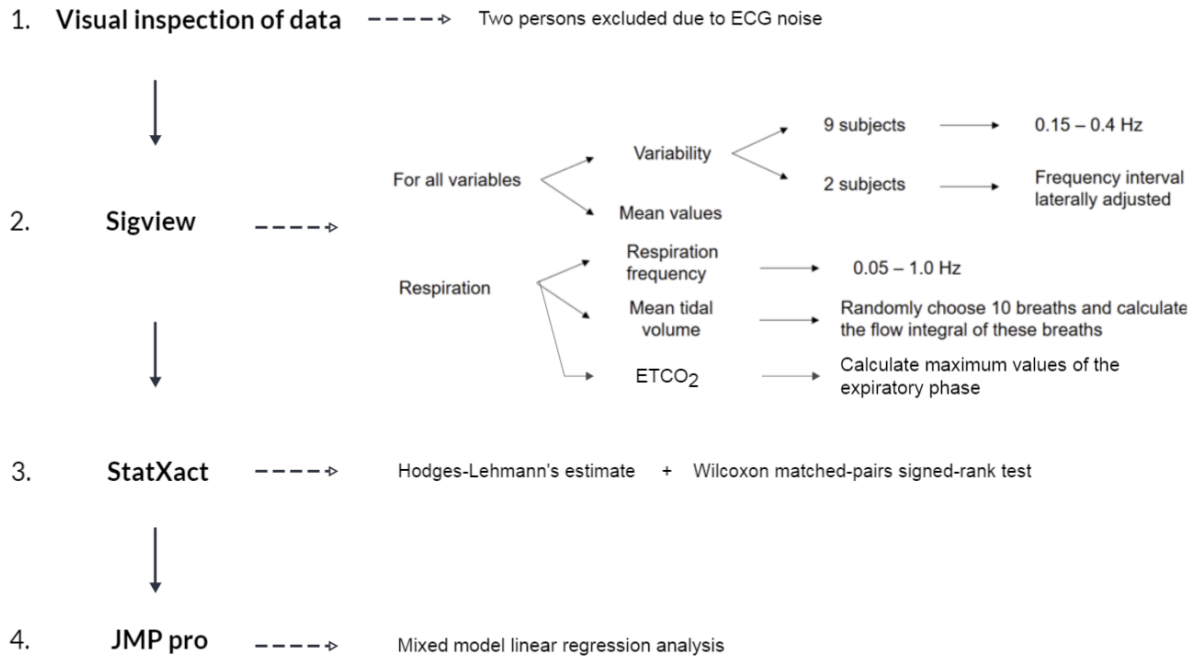

Figure 5: Overview of all the analyses that were done for this project thesis.

Results

I. Respiratory variables during mask air breathing and CO_2 breathing

The experimental protocol had a significant impact on respiratory variables. Tidal volumes exhibited a twofold increase from spontaneous to mask breathing and tripled from spontaneous to CO_2 breathing (table 1 in appendix). There was a slight decrease (by 0.04 Hz) in respiratory frequency during CO_2 breathing compared to spontaneous breathing. End-tidal CO_2 levels decreased marginally during mask breathing and increased significantly during CO_2 breathing, both in comparison to spontaneous breathing. All observed respiratory changes aligned with anticipated outcomes.

II. Cardiovascular variables during mask breathing and CO_2 breathing

CO_2 breathing resulted in statistically significant alterations in all cardiovascular variables except SV. The extent of change in HR, MAP, and RR-time during CO_2 breathing was consistently below 10% when compared to both spontaneous and mask breathing (table 1 in appendix). Therefore, the protocol elicited minimal shifts in overall hemodynamics.

III. Cardiovascular variability during mask breathing and CO_2 breathing variables

Breathing through a mask had an impact only on MAP variability, doubling in comparison to spontaneous breathing. Conversely, cardiovascular variability showed a significant increase during CO_2 breathing (Table 1 in appendix) compared to both spontaneous and mask breathing. In CO_2 breathing, variability in HR, in SV, and in RR-time all doubled, while MAP variability showed a tenfold increase from the levels observed during spontaneous breathing.

Discussion

Despite extensive studies on the functions of RSA and it's influencing factors, the physiological significance of RSA remains a subject of controversy (14, 18, 33). Nevertheless, several functions for RSA have been proposed such as stabilizing systemic blood flow and arterial blood pressure, and increasing pulmonary gas exchange efficiency.

One of the functions hypothesized for RSA is that RSA stabilizes systemic blood flow and arterial blood pressure by counteracting variations in arterial blood pressure associated with respiratory activity (34). Some studies have also illustrated that eliminating RSA led to increased arterial blood pressure variations (35, 36). Fluctuation in arterial blood pressure is identified as an independent factor contributing to end-organ failure (37).

To test this hypothesis, studies have used NIPPV and documented reduction of RSA with IPPV (38, 39). NIPPV disrupts the mechanical dynamics involved in the interplay between respiration and circulation, in addition to altering the intrathoracic pressure during respiration. IPPV also reduces the central feed-forward mechanism because the subject's involvement in initiating respiration is minimized (39). This overall leads to a reduction in RSA. Respiratory variability in MAP is higher during the use of IPPV compared to metronome breathing**.** Based on this, one could posit that the function of RSA is to stabilize cardiac output and blood pressure over the respiratory frequency (39).

Other studies claim that the function of RSA is to improve pulmonary gas exchange efficiency by matching alveolar ventilation and capillary perfusion throughout the respiration cycle. This matching spares the cardiac and respiratory energy by suppressing unnecessary heartbeats during expiration and ineffective ventilation during the waning phases of perfusion (23, 40, 41).

Hayano et al. (23). used anesthetized dogs to examine the implications of RSA. Hayano did this by imposing RSA, inverse RSA, and monotonic cardiac pacing at a fixed breathing rate; the study demonstrated that RSA enhanced gas exchange efficiency compared to monotonic and inverse RSA. Giardino et al (42) demonstrated that the amplitude of RSA was linked to improved gas exchange at low breathing rates. The suggested explanation for the improved gas exchange was that the increased heart rate during inspiration increased the blood flow through the pulmonary circulation when the alveoli were at peak oxygen concentration. The hypothesis

offered by Giardino et al was later disproved by scientists from New Zealand, where Sin et al proved that slow breathing alone was associated with improved gas exchange efficiency regardless of the RSA amplitude (43, 44). Sin et al also found that patients with cardiac pacemakers, and thus monotonic heart rates, had similar enhancements in ventilation efficiency during slow breathing exercises as subjects without pacemakers (and with intrinsic RSA) (45).

On the other hand, Ben-Tal et al (46) demonstrated in a theoretical study with a mathematical model that RSA did not improve gas exchange, but that RSA instead minimized the work rate of the heart while maintaining physiological levels of arterial CO_2. This shows the possibility of reducing energy expenditure while maintaining the same level of PaO_2. Lung ventilation is controlled to maintain a constant alveolar partial pressure of CO_2 (47). Both central and peripheral chemoreceptors increase the respiratory drive in reaction to hypercapnia, thereby inhibiting the vagal outflow during inspiration. Furthermore, slow, deep breathing also induces vagal nerve inhibition through pulmonary stretch receptors, contributing to RSA. In another study, Ben-Tal et al (47) suggest that partial pressure of O_2 in arteries (PaO_2) also influences the respiratory drive. Still, the magnitude of the drop in O_2 before influencing lung ventilation must be relatively large.

Respiration influences the circulatory system. During inhalation the intrathoracic pressure decreases, facilitating air to enter into the lungs. Simultaneously, the intra-abdominal pressure rises, increasing blood flow into the major veins in the thoracic cavity. This results in increased right ventricular stroke volume during inspiration (34). Nevertheless, the increased filling of the right ventricle hinders the filling of the left ventricle due to their shared myocardium and are located within the pericardial sac. Consequently, an increase in right ventricular diastolic pressure is transmitted to the left ventricle, reducing the filling rate in these low pressure system (48). This corresponding adjustments in left cardiac stroke volume, coupled with the respiratory driven changes in hear rate, results in nearly constant cardiac output from the left cardiac ventricle.

In our study we found that an increase in tidal volume was associated with an increase in RSA, this phenomenon has also been observed in other studies (49, 50). When tidal volume increases and the respiratory rate decreases, there are observable increase in heart rate and blood pressure fluctuation. These fluctuations could be due to pulmonary stretch receptors, arterial baroreceptors, and central respiratory drive (49).

Elevated levels of $ETCO_2$ are associated with respiratory conditions or changes in ventilation. An increase in $ETCO_2$ can alter the acid-base balance in the blood, and thus influence chemoreceptors sensitive to CO_2 levels. Since the respiratory and cardiovascular system are closely linked, alternations in respiratory pattern can lead to changes in heart rate.

Limitations

In our study, we observed that elevated levels of both end-tidal CO_2 and tidal volumes correlated with an augmentation in high-frequency HRV. Unfortunately, we were unable to differentiate the extent to which elevated levels of both end-tidal CO_2 and increased tidal volumes contributed to HRV. Our study included only thirteen healthy subjects. This small sample size may not adequately represent the broader population, limiting the generalizability of our findings. Two subjects had to be removed because of noise in the ECG, and the introduction of NIPPV and CO_2 in the inhaled air may be perceived as uncomfortable, and we are uncertain whether this could have influenced the results. We also used fast Fourier transform during the analyses, which can also result in biases because of artificial interpolation.

Conclusion

In summary, our study explored the intricate interplay between the cardiovascular and respiratory systems, examining the regulation of heart rate and blood pressure. Fluctuations are present in the majority of biological systems. A notable portion of HRV stems from interaction between the cardiovascular and respiratory system.

One significant phenomenon explored was RSA, a manifestation of HRV in synchrony with respiration. While RSA's physiological significance remains debated, it might contribute to stabilizing systemic blood flow and arterial blood pressure, countering variations linked to respiratory activity. Additionally, some studies propose that RSA aims to enhance pulmonary gas exchange efficiency.

Examining the impact of short-term high-frequency HRV and RSA in response to elevated CO_2 levels, our findings suggest a notable increase in RSA with rising TV and $ETCO_2$.

Acknowledgements

Maja Elstad, Maria Skytioti, Lars Walløe, Signe Søvik and Julia K. Gundersen played important roles in the completion of this thesis. We sincerely appreciate their contributions and support.

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Appendix

TABLE I RESPIRATORY AND CARDIOVASCULAR VARIABLES DURING SPONTANEOUS BREATHING, MASK BREATHING, AND CO2 BREATHING IN HEALTHY SUBJECTS.

Table I	**Conditions**		
	Spontaneous Breathing	**Mask Breathing**	***CO_2 Breathing***
Resp. Freq (Hz)	0.25 (0.21 – 0.31)	0.21 (0.17 – 0.26)	0.21* (0.16 – 0.26)
Tidal Volume (mL)	525 (427 – 633)	1104 (737 – 1421)	1706 *# (1126 – 3367)
ET-CO_2 (mmHg)	35.7 (33.8 – 37.6)	34.7 * (32.0 – 36.7)	38.4 *# (37.4 – 39.2)
HR (bpm)	58.9 (53.8 – 64.0)	58.7 (53.8 – 63.8)	62.9 *# (56.2 – 69.1)
SV (mL/beat)	104 (87 – 120)	106 (88 – 123)	107 (89 – 125)
MAP (mmHg)	71.3 (66.5 – 76.8)	71.6 (67.9 – 76.9)	76.2 *# (72.1 – 80.0)
RR-time (ms)	1034 (950 - 1128)	1037 (952 - 1136)	979 *# (877 - 1080)

Table 1: Medians with their 95% confidence intervals are calculated by Hodges-Lehmann's estimate for one-sample. For comparisons between the three different conditions, Wilcoxon signed- rank test was used. *Resp. Freq* respiration frequency, *ET-CO_2* endtidal-CO_2, *HR* heart rate, *SV* left cardiac stroke volume, *MAP* mean arterial blood pressure, RR-time duration between two R peaks in the electrocardiogram. n = 11.

* $P \leq 0.05$ when compared with spontaneous breathing

$P \leq 0.05$ when compared with mask breathing

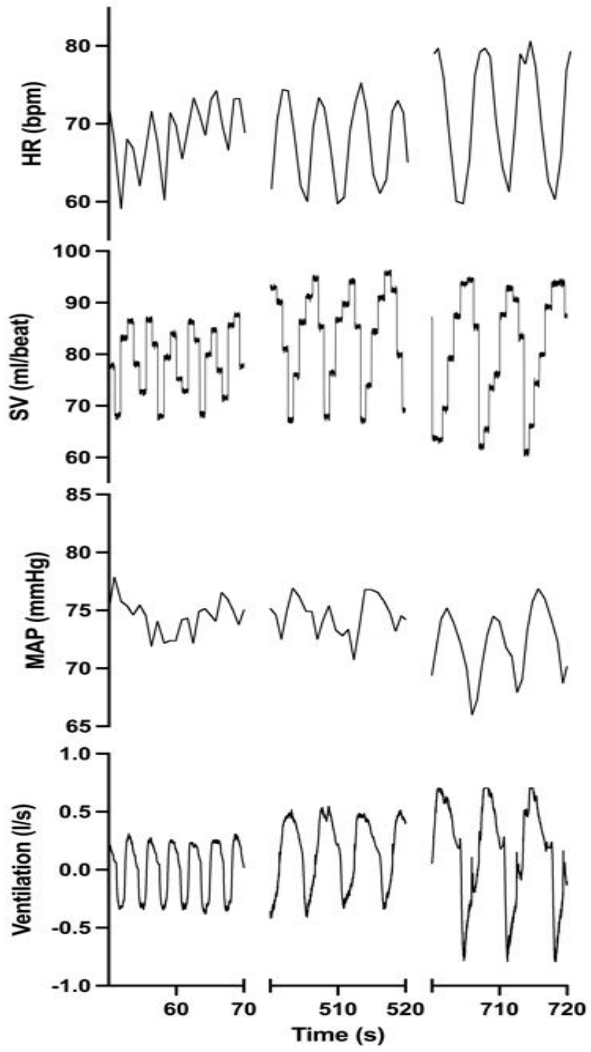

Figure 6. Recordings of heart rate (HR), cardiac stroke volume (SV), mean arterial pressure (MAP), and ventilation from one representative subject.

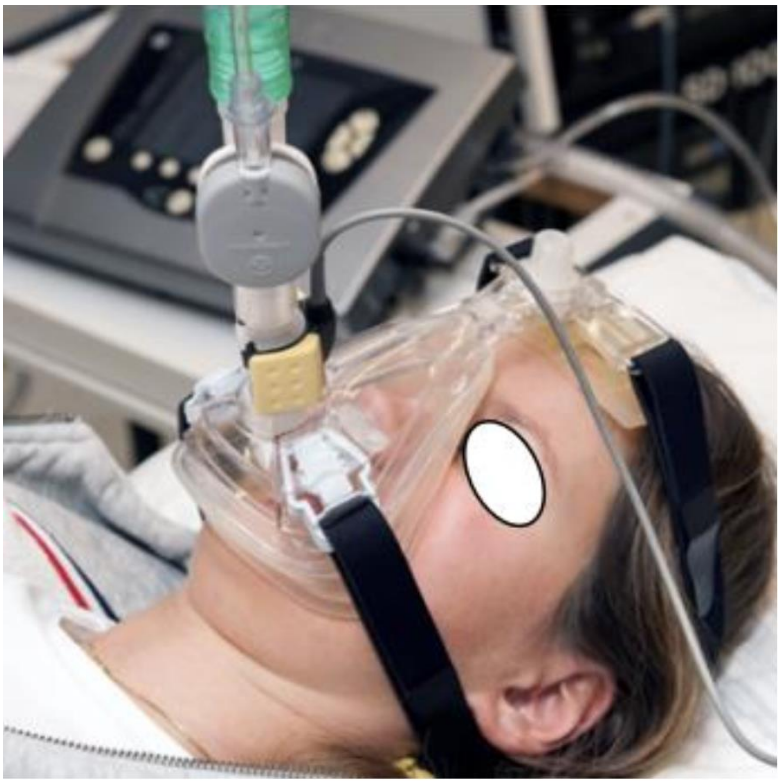

Figure 7. Non-invasive mechanical ventilation.

Extended abstract

High frequency heart rate variability was increased by addition of 5% CO_2 to breathing air in healthy, young humans

Atiq M. Ayoubi*[1], Yasin A. Hassan*[1], Nathalie L.A. Holme[1], Agata S. Koffeld[1,2], Marian B. Andersen[1], Signe Søvik[2], and Maja Elstad[1]

***Abstract*— Oscillations in cardiovascular variables may be part of the cardiovascular control mechanisms. Heart rate variability in the high frequency interval is part of the cardiorespiratory interactions. Main part of high frequency heart rate variability is called respiratory sinus arrhythmia, which is influenced by ventilation pattern. We investigated how addition of 5 % CO_2 to the air affected the high frequency heart rate variability. CO_2 breathing increased heart rate variability, and this was linearly related to increases in both tidal volume and end-tidal CO_2. CO_2 breathing also increased heart rate, which did not affect heart rate variability.**

I. INTRODUCTION

The human cardiorespiratory system displays a vast variety of oscillations. Heart rate variability is one of the most studied oscillations. One of the causes of heart rate variability is respiratory sinus arrhythmia (RSA). RSA is a physiological phenomenon where the heart rate increases during inspiration and decreases during expiration. RSA is present in young adults and decreases with age and in diseases such as depression, autoimmune diseases, hypertension, and heart failure [1]. Several physiological mechanisms contribute to RSA. These mechanisms include central neural networks, peripheral feedback mechanisms and intrinsic mechanisms [2]. In this study, we wanted to investigate how an increase in partial pressure of arterial carbon dioxide ($PaCO_2$) - which in turn activates chemoreceptors - affects RSA. We hypothesized that an increase in $PaCO_2$ would increase the amplitude of RSA.

II. MATERIALS AND METHODS

A. Subjects

Thirteen young, healthy volunteers of both genders (5 males, 23 years [range 19-28], body mass index 23 kg/m2 [19-26]) participated in the study after written informed consent. None of the subjects were taking any medication (except contraceptive pills) and all were non-smokers. The study was approved by the Regional committee for Medical and Health Research Ethics.

B. Experimental protocols

The part of the experimental protocol presented in this abstract lasted 15 minutes (Fig. 1). Each subject lay supine on a bench, and the recordings started with the subject breathing room air spontaneously through an open ended facemask. After 5 minutes of spontaneous breathing, the facemask was connected to a breathing circuit on a ventilator (Maquet Servo-*i*) set at minimum positive end expiratory pressure (PEEP) (2 cmH2O) and zero pressure support. For 5 minutes the subjects spontaneously breathed medical air provided by the ventilator, then 5% CO_2 mixed in air was provided in the circuit for 5 minutes.

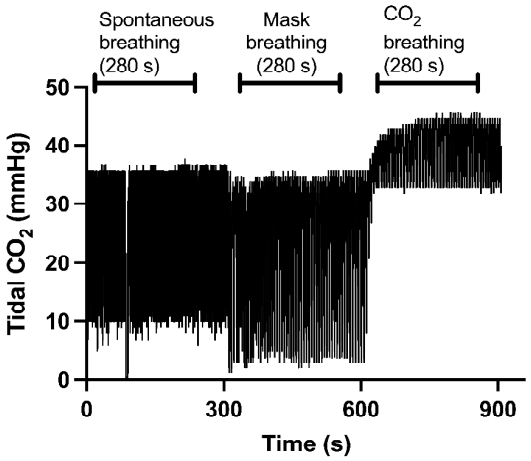

Figure 1. Study protocol displaying one subjects´ breathing conditions. The protocol started with 5 min (~ 300 seconds) of spontaneous breathing, then 5 min of mask breathing and ended with 5 min of CO_2 breathing.

III. MEASUREMENTS AND ANALYSES

We recorded three-lead electrocardiogram (ECG) (300 Hz sampling rate, SD-100, Vingmed, Horten) and finger arterial blood pressure wave (Finometer, FNS, The Netherlands). Respiratory air flow was measured by a flow head (Hamilton Medical) connected to a custom-made differential pressure transducer. Tidal capnography was recorded from a nasal cannula. From the ECG, the time distance between two R peaks was the RR interval, and heart rate (HR) was calculated beat-by-beat as 60/RR interval. We estimated mean arterial blood pressure (MAP) from the blood pressure wave. Left cardiac stroke volume (SV) was estimated by the Finometer (ModelFlow algorithm [3]).

*Shared first authorship.

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Other data than ECG was sampled at 100 Hz into a recording computer (Regist, Morten Eriksen, Oslo, Norway).

A. Analysis

Two of the subjects had noise in the ECG recording during CO_2 breathing, and were omitted before the statistical analysis. The mean value and variability of every respiratory and cardiovascular variable for each individual were calculated (SIGVIEW, version 5.3.2, http://www.sigview.com). The data recorded were analyzed in 280 s time epochs from the three conditions.

Variability of cardiovascular variables were calculated using fast Fourier transform spectral analysis. Power spectral density integrals were calculated for the high frequency interval 0.15-0.4 Hz for all three conditions in nine of eleven subjects. In the two remaining subjects, the frequency interval was adjusted laterally to cover their respiratory frequency. The respiratory frequency was estimated from the peak in the frequency power spectra of respiratory activity within 0.05-1.0 Hz. Mean tidal volume was calculated by randomly choosing 10 breaths for each condition and calculating the flow integral of these breaths.

B. Statistical analysis

We calculated the medians and their 95 % confidence intervals by Hodges-Lehmann estimate [4], and for the testing of the differences between conditions the Wilcoxon matched-pairs signed rank test against a two-sided alternative was used [4] (StatXact, Cytel Studio 10, Cytel Inc., Cambridge, MA, USA). Mixed model multiple linear regression was performed in JMP Pro 16 (jmp.com).

IV. RESULTS

A. Respiratory variables during mask air breathing and CO_2 breathing

The experimental protocol influenced the respiratory variables markedly. Tidal volumes doubled from spontaneous to mask breathing and tripled from spontaneous to CO_2 breathing. The respiratory frequency decreased slightly (by 0.04 Hz) during CO_2 breathing compared to spontaneous breathing. End-tidal CO_2 decreased marginally during mask breathing and increased substantially during CO_2 breathing, both in comparison to spontaneous breathing. All respiratory changes were as expected.

B. Cardiovascular variables during mask breathing and CO_2 breathing

Although CO_2 breathing induced statistically significant changes in all cardiovascular variables except SV, the magnitude of change in HR, MAP and RR-time during CO_2 breathing were all less than 10% compared to spontaneous and mask breathing. The protocol thus imposed minimal changes in overall hemodynamics.

C. Cardiovascular variability during mask breathing and CO_2 breathing variables

Mask breathing only affected MAP variability, which was doubled compared to spontaneous breathing.

Cardiovascular variability was however markedly increased during CO_2 breathing (Table 1) as compared to both spontaneous and mask breathing. During CO_2 breathing, variability in HR, SV and RR-time doubled, while MAP variability had a tenfold increase from spontaneous breathing.

TABLE I HIGH FREQUENCY VARIABILITY OF CARDIOVASCULAR VARIABLES DURING SPONTANEOUS BREATHING, MASK BREATHING AND CO_2 BREATHING IN HEALTHY SUBJECTS.

Table I	**Conditions**		
	Spontaneous Breathing	**Mask Breathing**	***CO_2 Breathing***
HRV_{HF} (bpm^2)	7.0 (3.0 – 12.0)	10.2 (3.2 – 17.0)	23.9 $^{*\#}$ (11.7 – 33.9)
SVV_{HF} (mL^2)	21.5 (12.8 – 29.7)	27.7 (13.5 – 41.9)	50.3 $^{*\#}$ (30.8 – 80.6)
$MAPV_{HF}$ ($mmHg^2$)	0.30 (0.18 – 2.02)	0.67 * (0.31 – 3.30)	3.10 $^{*\#}$ (0.88 – 7.04)
RRV_{HF} (ms^2)	1694 (844 – 5218)	2581 (881 – 5440)	5613 $^{*\#}$ (3085 – 8467)

Medians with their 95% confidence intervals are calculated by Hodges-Lehmann's estimate for one-sample. For comparisons between the three different conditions, Wilcoxon signed-rank test was used. *HRV* heart rate variability, *SVV* left cardiac stroke volume variability, *MAPV* variability of mean arterial blood pressure, *RRV* variability RR-time. $n = 11$. Variability calculated in the high frequency interval. * $P \leq 0.05$ when compared with spontaneous breathing $^{\#}$ $P \leq 0.05$ when compared with mask breathing

In mixed model multiple regression of all experimental conditions in all eleven subjects, variability in HR was positively associated with level of end-tidal CO_2 and tidal volume (both $p<0.01$), but was unaffected by HR ($p=0.62$) (Fig. 2).

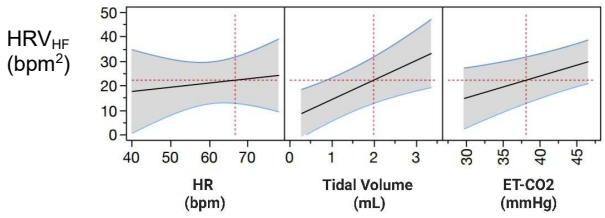

Figure 2. Heart rate, tidal volume, and end tidal CO_2 effect on heart rate variability in all eleven subjects at the three experimental conditions.

V. CONCLUSION

We found that increases in both end-tidal CO_2 and tidal volumes led to an increase in high frequency HR variability. Supraphysiological increase in $PaCO_2$ increases RSA amplitude, but we are cautious about concluding on the effect of $PaCO_2$ when within physiological limits.

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