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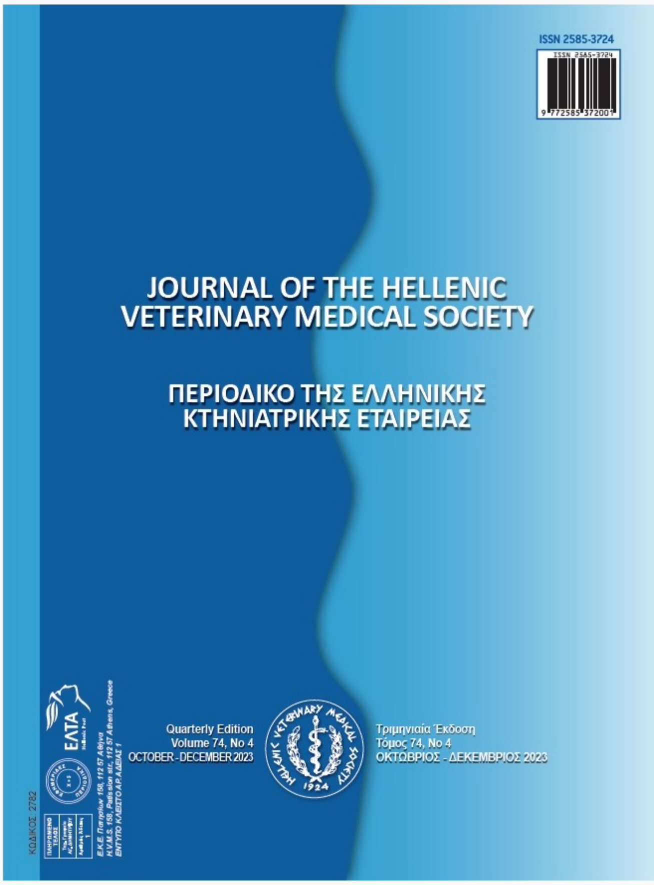

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The effects of mechanical ventilation on heart rate variability and complexity in mice

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ABSTRACT: In a variety of diseases, altered respiratory modulation is often as an early sign of autonomic dysfunction. Therefore, understanding and evaluating the effects of mechanical ventilation on the autonomic nervous system is vital. The effects of mechanical ventilation on autonomic balance have been assessed by heart rate variability (HRV) using frequency domain and non-linear analysis including fractal complexity and entropy analysis in anesthetized mice. BALB/c mice (n=48) were divided into two groups: Spontaneous breathing and mechanical ventilation. The electrocardiograms were recorded. Four different types of analysis were employed: i. frequency domain analysis, ii. Poincaré plots, iii. Detrended Fluctuation Analysis (DFA) and iv. Entropy analysis. An unpaired t-test was used for statistical analysis. In a ventilated group, very low frequency (VLF) and low frequency (LF) parameters were not changed, whereas the high frequency parameter was decreased compared to spontaneous breathing mice. DFAα_1 was significantly increased due to mechanical ventilation but DFAα_2 was unchanged. In Poincaré plots analysis, standard deviation 2 (SD2) / standard deviation 1 (SD1) ratio was increased, however, SD1 and SD2 were not significantly affected. Also, Approximate Entropy and Sample Entropy remained unchanged. HF parameter, DFAα_1, and SD2/SD1 were affected by mechanical ventilation. Decreased HF and increased DFAα_1, further support the notion that HRV is dominated by respiratory sinus arrhythmia at high frequencies, this may be due to decreased vagal tone caused by mechanical ventilation. This novel results of HRV analysis are important considering increased usage of HRV techniques day by day in animal models and other medical practices.

Keywords**:** mice, heart rate variability, mechanical ventilation, heart rate complexity

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INTRODUCTION

Mechanical ventilation has substantial position considering that more than 200 million patients require respiratory support for surgery under general anaesthesia (Weiser *et al.*, 2008). Up to 20 million patients during their admission to Intensive Care Units (ICUs) annually require mechanical ventilation (Ambrosino and Vitacca, 2018). The outbreak of COVID-19 pandemic has dramatically increased the number of patients who need respiratory care (Iyengar *et al.*, 2020). In a variety of diseases, altered respiratory modulation is often an early sign of autonomic dysfunction (Bernardi *et al.*, 2001). Therefore, understanding and evaluating effects of mechanical ventilation on autonomic nervous system (ANS) is vital. The ANS regulates physiological control mechanisms in response to any homeostatic change. A direct evaluation of the ANS activity is very challenging. Heart rate variability (HRV) is a reliable tool for the assessment of the ANS functions (Schmidt *et al.*, 2005). Therefore, analysis of different parameters of HRV may allow to monitor the changes in the functions of ANS during important processes that the patients go through such as weaning from mechanical ventilation (da Silva *et al.*, 2023). However, to our knowledge, there are limited amount of studies that focus on fractal complexity of heart rate (HR) series under mechanical ventilation in the literature. Therefore, in the present study, we have focused on possible effects of mechanical ventilation on complexity in heart rate in anesthetized mice. The analysis method used for beat-to-beat heart rate alternations have key importance considering different numerical methods may provide different results. Thus, we have applied four different numerical analysis for a comparative evaluation: i. frequency-based analysis of HR which is the most used in the literature and referred to as heart rate variability analysis (HRV), ii. detrended fluctuations analysis (DFA) of HR series, iii. Poincare plots; standard deviation 1 (SD1) and standard deviation 2 (SD2) and the ratio of SD2/SD1, iv. Entropy analysis.

The HRV is a numerical analysis technique that gives the power of heart rate changes that lie within certain frequency bands (Tarvainen *et al.*, 2014): Researchers have identified that certain frequency bands correlate with certain physiological changes (Malik *et al.*, 1996).

In contrast to frequency analysis of the heart rate (HRV), DFA, Poincare plots and Entropy analysis are non-linear analysis methods which have not been used very often, (Shaffer and Ginsberg, 2017). The DFA analysis deals with "non-periodical" and or "quasi-periodical" alternations of the beat-to-beat heart rate changes (Penzel *et al.*, 2003). The cardiac rhythm exhibits complexity as the indicator of a complex interaction between the pacemaker cells of the heart and the ANS (Goldberger *et al.*, 2002). DFA has been shown to predict cardiovascular outcomes accurately and has been recommended to predict morbidity and mortality for several diseases (Magrans *et al.*, 2013). DFA is a method that calculates the long-term correlations and fractal structure in heart rate dynamics. DFA calculates the scaling exponents (short-term and long-term) from the time series and exposes fractal correlation properties of complex heart rate series (Sassi *et al.*, 2015). Although the frequency domain analysis, so called HRV is reliable in some groups (Dabas and Shaw, 2010), however, it may be insufficient to explain the complexity of heart rate dynamics (Peng *et al.*, 1995). The Poincaré plot as " a practical non-linear analysis method" has also been used to evaluate the autonomic modulation and especially the randomness of heart rate (Guzik *et al.*, 2007) although it was not well valued.

Entropy analysis have been integrated into the complexity testing research to determine the disorder status of data dynamics. Entropy can be mathematically defined as a negative natural logarithm of a conditional probability. A higher value implies a higher complexity of time series, with a lower likelihood that the similarity will continue as the embedding dimension grows (or a grater probability that similarity changes). The Approximate Entropy (ApEn) and Sample Entropy (SampEn) algorithms are the most popular ones (Mandelbrot and Aizenman, 1979).

Originally, approximate entropy was devised as a functional application of a nonlinear dynamical system's Kolmogorov-Sinai entropy. It may also be an approximation of a method's differential entropy rate. More recently, to enhance ApEn, SampEn has also been developed. Sample entropy converges quite faster at the expense of a greater variance of the estimates (when calculated on a shorter series).

As well known, breathing is the major parameter causing heart rate to fluctuate which further reflects on the HF parameter in the frequency domain analysis (Song and Lehrer, 2003). A prominent respiratory dependent HR change is called as respiratory sinus arrhythmia (RSA), which may be a sign of increased vagal efferent activity on HR (Malliani *et al.*, 1991).

Accordingly, it was considered that HF parameter in the frequency domain analysis represents vagal activity mainly due to spontaneous breathing or RSA (Schipke, 1999). It was proposed that cardiorespiratory center inhibits vagal efferent outflow which accelerates the HR during spontaneous inspiration and "vagal influence" returns to normal level slowing down the HR during spontaneous expiration (Eckberg, 1983). Another proposed mechanism for respiratory dependent HR fluctuations is that the increase in HR during inspiration is dependent on the increased "venous return" induced by decreased intrathoracic pressure (Rothe, 2011). In summary, both "vagal influence" and "venous return" may increase the heart rate during inspiration and decrease HR during expiration. Therefore it can be stated that the characteristics of every breath may be influenced by various reflexes (Corne and Bshouty, 2005). It is hypothesized that mechanical ventilation might trigger these reflexes and decrease the vagal motor output generated by the breathing centers (Bartlett and St. John, 1988).

The aim of this study is to assess not only interactions between autonomic nervous system and spontaneous/mechanical ventilation but also to detect the parameters that are affected by mechanical ventilation.

METHODS

Animals and Anaesthesia

The in-vivo mice experiment of the present study was performed with the permission of local "Ethics Committee for Animal Experimentations" (No:77.637.435-04) and in accordance with the Guide for the Care and Use of Laboratory Animals. 10-12 weeks old, 48 male BALB/c mice weighing 25 ± 2.8 grams were purchased from the KOBAY Incorporated Company (Ankara/Turkey). We preferred to use male BALB/c mice because it has been reported that the menstrual cycle effects HRV analysis (Sato *et al.*, 1995). The animals were housed for at least five days in a special room with a room temperature of 20-22 °C under the 12-hour light-dark cycles in the animal care center. Drinking water and rodent pellets were provided ad libitum. All experiments were performed at daytime.

Just before experiments, each mouse was weighed for the calculation of the anaesthetics/analgesics dose. The mice were anesthetized with intraperitoneal injection of Na-Pentobarbital (90 mg.kg-1, i.p.) (SIGMA Inc. Germany), and an additional, use of Fentanyl (0,2 mg.kg-1, i.p.) (GENESIS Inc. Istanbul-Turkey) in order to avoid pain. The pedal withdrawal reflex and the breathing frequency were both used to assess the depth of anaesthesia during the procedure and when needed 25 percent of the initial dose was administered to prolong the anaesthesia. Animals were placed on a thermal plate in order to avoid hypothermia due to the anaesthetic and analgesic used. The rectal temperature of the animals was observed during experiments and kept around 36,5 ºC.

Tracheostomy Procedure

Tracheostomy procedures were performed according to the model described by Alluri et al. (2017) (Alluri *et al.*, 2017). A 1 - 1.5 cm longitudinal midline incision just below the larynx was implemented with a curved fine scissors. The facial membrane between the glands were penetrated by a blunt dissection with 2 curved serrated forceps in order to expose the trachea. A metal cannula was placed into the trachea and connected to the mechanical ventilator with very small death space, highly accurate, positive pressure pump developed specially for small laboratory animals (Ozbek, 2002).

Experimental Design, Sham Procedure, Spontaneous breathing and mechanical ventilation

In order to evaluate effects of mechanical ventilation on the heart rate dynamics and complexity in BALB/c mice, we have constructed two groups depending on the ventilation type. A total of 48 mice were divided into two groups: the spontaneous breathing group (n=24) and mechanical ventilation group (n=24).

The same procedural steps explained above were followed in both groups but the metal cannula was only connected to mechanical ventilator in mechanical ventilation group. This eliminated any procedural differences between the spontaneous breathing group (sham group) and mechanical ventilation group that could have affected the results. In spontaneous breathing group the frequency of spontaneous breathing was between 70 and 80 min^{-1}.

In the mechanically ventilated group, a constant pressure ventilator was used. In the case of mechanical ventilation inspiratory pressure was 15± 1.66 cm H_2O. The respiratory frequency was adjusted to 72 min^{-1} whereas inspiration expiration ratio was 1 to 2. The study protocol is summarized in Figure 1.

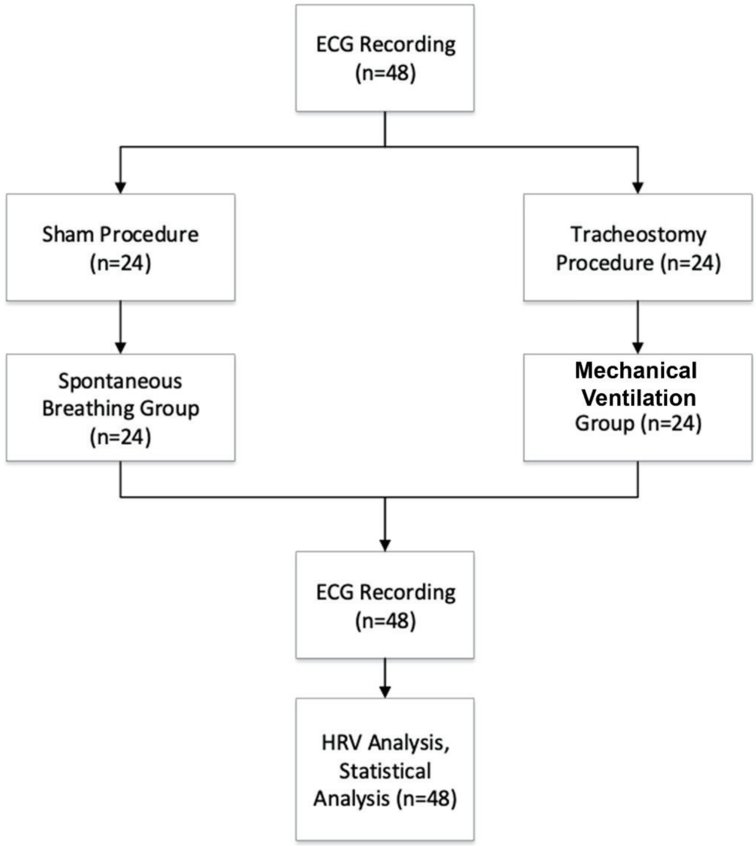

Figure 1. A Schematic diagram of the group design.

The breathing frequency adjustment of mechanically ventilated group was made according to the literature (Ewald, Werb and Egeblad, 2011) and to our observation of spontaneous breathing rate under Na-pentobarbital anesthesia. The mechanical ventilation frequency of 1.2 Hz also remains within the frequency of HF band, which is important for frequency domain analysis of HRV.

After completing the procedures in both groups, the animals were placed in a supine position on a thermal plate for 60 minutes of electrocardiography (ECG) recording. The entire experiment, including the procedures and ECG recordings, lasted approximately 90 minutes. Following the ECG recordings, the animals were humanely euthanized using the cervical dislocation method under anesthesia.

ECG recordings

The needle ECG electrodes were placed under skin of right arm and left leg for the Lead II. Surface ECGs were recorded by using Powerlab/SP8 (AD Instruments, Australia). ECG sampling frequency was adjusted to 4k Hz. The high-pass and low-pass filter settings were 0,3 Hz and 1 kHz, respectively, no notch filter was required. LabChart 7 software (AD Instruments, Australia) was used for “R” wave detection (see Figure 2.).

Frequency Domain and Non-linear Analysis of HR series

R waves were detected by setting threshold value using Pan-Tompkins real-time QRS detection algorithm (Singh, 2010), then the tachogram of RR intervals was obtained. These RR tachograms were transformed to time series by using Berger interpolation. Same data sets were used for all of the analyses, namely the same time series used for the frequency domain and non-linear analysis.

Frequency Domain Analysis

Kubios HRV Software (University of Eastern, Finland) was used for the HRV analysis. Each recording period was represented by four minutes of R-R tachogram and they were re-sampled with 10 Hz to convert to time series.

The frequency bands of our analysis are selected as proposed by Thireau et al. (2008) (Thireau *et al.*, 2008), LF and HF bands were modified due to me-

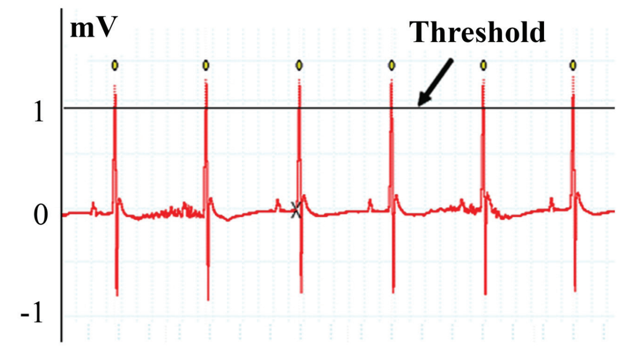

Figure 2. "R" wave detection.

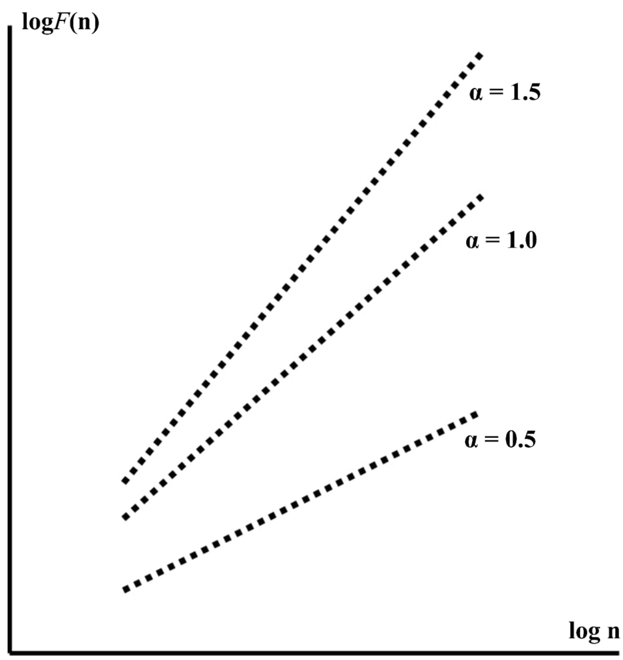

Figure 3. Schematic presentation for the log-log plot of fluctuation function F(n). Slope of the logF(n) trend was characterized to correlation properties of HR signal. An α = 0.5 indicates white noise (uncorrelated-random data). An α greater than 0.5 and less than or equal to 1.0 specifies determined long-range power-law correlations. An α = 1 is parallel to the 1/f noise whereas α = 1.5 corresponds to brown noise (Haddadian *et al.*, 2013). It is generally acknowledged that fractal like fluctuations, which are typical of healthy physiological control, range between 0.75- 1.25 (Delignières and Marmelat, 2012).

chanical ventilation frequency selection of our study so as to include mechanical ventilation frequency in HF band. The bandwidth chosen were as follows: VLF: 0,00-0,15 Hz, LF: 0,15-1,0 Hz, HF: 1,0-5 Hz.

Ultra-low frequency (ULF) and very high frequency (VHF) parameters were not defined for mouse therefore were not included in this study, LF/HF ratio was also evaluated additionally. Percentage of power spectrum densities (%PSD), namely relative powers of frequency bands were documented.

Detrended Fluctuations Analysis (DFA)

We analyzed RR time series according to DFA algorithm introduced by Peng (Peng *et al.*, 1995), using Kubios HRV Software (University of Eastern, Finland). This method uses the detrending approach to better examine the fluctuations in time series. Scaling exponents obtained as a result of this analysis reflects the relationship between the fluctuation function and the sample size. This relation between them is an expression of the self-similarity of the time series. This method consists of several steps: Firstly, the integrated time series y (k) is obtained from the sum of the differences between the average RR (RR_a) and each RR value (RR_i) in the time series (Eq1).

$$y\,(k) = \sum_{(i=1)}^{k} [RR_i - RR_a] \qquad (1)$$

The integrated time series y (k) is divided into non-overlapping, equal sized (n) boxes. For the detrending of integrated time series, the local trend y_n (k) is calculated for each box and then detrended by the subtracting from integrated time series y (k). Fluctuation function F (n) is calculated by the root-mean-square of the detrended time series obtained from the previous step as follows equation (Eq2);

$$F\,(n) = \sqrt{\frac{1}{N}\sum_{(k=1)}^{N} [y\,(k) - y_n\,(k)]^2} \qquad (2)$$

Where F (n) is the fluctuation function of box size n, N is the number of the value in the time series, y (k) is the integrated time series, y_n (k) is the local trend series in each box.

The logarithmic relationship between the fluctuation function F (n) and the box size n is scaled to determine the correlation properties of the time series. The scaling exponent $DFA_{\alpha 1}$ and $DFA_{\alpha 2}$ are obtained from calculation the slope of this logarithmic relation (see, Figure 3.).

In this study, short-term ($DFA_{\alpha 1}$) and long-term ($DFA_{\alpha 2}$) scaling exponents were calculated for different box sizes 4<n<11 and 12<n<64 respectively in both groups (Lin *et al.*, 2016).

Poincaré plot analysis

The analysis of the Poincaré plot was performed with the use of the Kubios HRV Software (University of Eastern, Finland) by drawing an ellipse to the plotted points. From the analysis point of view, Poincaré plot is a quantitative visual analysis technique. The correlation spectrogram attained by Poincaré plot allows a compact representation of the time series regardless of the longitude of the time series. Each pair of RR intervals (previous and next) are placed in the rectangular coordinate system according to their coordinates (x, y), where x is the value of the RRn interval and y is the value of RRn + 1 (Tayel and Al-Saba, 2015). The dispersion of the graph provides a section of points whose center is located on the line called "line of identity". The identity line is the graph of the function x = y (RRn = RRn+1). Points above the line of identity means that RR intervals that are longer than the previous one, and points below the line of identity means a shorter RR interval than the preceding RR interval (Roy, Goswami and Sengupta, 2020).The following 3 parameters are calculated using these coordinates: (i) SD1, (ii) SD2 and (iii) SD2/SD1 which were included in the present study.

(i) **SD1**, the standard deviation of the distance of each point from the y= x axis, defines the ellipse's width. SD1 was considered to be correlating with blood pressure changes, and power in the LF and HF bands, and total power of frequency domain analysis obtained from short-term recordings of 5 minutes (Zerr *et al.*, 2015).

(ii) **SD2**, the standard deviation of each point from the y= x + average R-R interval, defines the ellipse's length. It has been hypothesized that SD2 reflects LF band power and baroreflex sensitivity (Brennan, Palaniswami and Kamen, 2002).

(iii) **SD2/SD1** ratio was considered as being the analog of LF/HF ratio from frequency domain analysis (Guzik *et al.*, 2007).

Entropy Analysis

The analysis of the entropy was performed with the use of the Kubios HRV Software (University of Eastern, Finland). Approximate entropy and SampEn measure the probability that in a given sequence of length N, runs of templates that are close to m points remain close (less than a certain tolerance level r) to m + 1 points. For the parameter selection of r and m, there is no fail-proof rule. Typically, values between 10 and 25 percent (usually 20 percent) of the standard HRV deviation are used. M = 2 is often used for template length; m = 1 is used for very short time series (Sassi *et al.*, 2015).

The regularity and complexity of a time series are calculated by estimated entropy. ApEn is intended for short time series in which there might be any noise and does not give any information about the dynamics of the underlying mechanism. Large ApEnvalues, applied to HRV data, imply poor predictability of fluctuations in successive RR intervals. Small values of ApEn mean the signal is periodic and predictable (Shaffer and Ginsberg, 2017).

A less biased and more accurate calculation of signal accuracy and complexity was developed to provide sample entropy. SampEn values are translated and used as ApEn and can be determined from less than 200 values in a much shorter time series (Shaffer and Ginsberg, 2017)

Statistical Analysis

The differences of mean HRV parameters between spontaneous breathing group (sham group) and mechanically ventilated group (study group) were compared using unpaired student's t-test. To do so, we applied the Shapiro-Wilk test to check whether the data are normally distributed or not. Since our data seem to be normally distributed according to Shapiro-Wilk, further analysis of our data was carried out by parametric statistical method. For statistical analyses, IBM SPSS Statistics Version 21.0 (SPSS Inc., Chicago, IL, USA) were used and $p<0,05$ was accepted as statistically significance level.

RESULTS

Frequency Domain Analysis

To eliminate variations between animals' magnitude of heart rate fluctuations we preferred to evaluate the relative power of frequency bands defined in methods. Therefore, the relative powers of heart rate frequency band comparisons between groups were as follows: Mean VLF parameters in ventilated group was significantly increased from 45,29% ± 23,24%

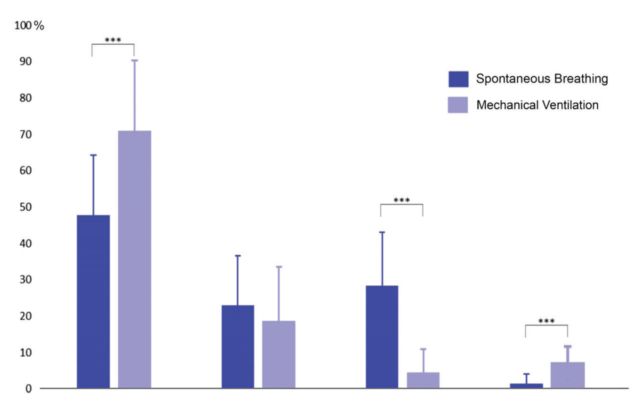

Figure 4. The relative powers of frequency domain analysis in both spontaneous breathing and ventilated group. ***: significantly different, $p<0,001$, compared to spontaneous breathing.

to 56,02% ± 37,52% ($p<0.05$). In contrast, mean LF power was not changed, 19,77% ± 19,07% vs 27,99% ± 31,04% ($p>0.05$), whereas mean HF power was dropped drastically from 28,30% ±18,23 to 15,99% ± 24,05 ($p<0,0001$). In parallel with this finding the mean ratio of LF/HF was found to be elevated in ventilated group when compared to spontaneous breathing group from 1,33 ± 3,05 to 4,20 ± 6,65 ($p>0.05$), see Figure 4.

Detrended Fluctuations Analysis (DFA)

Detrended Fluctuations Analysis scaling exponents, DFAα_1 (short-term) and DFAα_2 (long-term), were compared between the two groups:

Figure 5a includes representative results obtained from a spontaneous breathing and an mechanically ventilated animal. For each animal, α_1 and α_2 scaling exponents are illustrated separately to visualize the difference in slopes. The mean values of short term and long-term scaling exponents, DFAα_1 and DFAα_2, are shown in Figure 5b. Mean DFAα_1 were calculated in spontaneous breathing and mechanically ventilated group as 0,503 ± 0,24 and 0,729 ± 0,29, respectively. There was a statistically significant increase ($p<0,01$) in DFAα_1 in mechanically ventilated group when compared to spontaneous breathing group, see Figure 5b. Mean DFAα_2 was calculated as 0,901 ± 0,26 and 0,951 ± 0,28 in the spontaneous breathing and mechanically ventilated groups, respectively. The difference was not statistically significant.

Poincaré plot analysis

In Poincaré plots, SD1, SD2 parameters and SD2/SD1 ratio were compared between the two groups. Figure 6a illustrates two typical Poincaré plots belonging to spontaneous breathing and ventilation groups. In the spontaneous breathing group, RR (n) vs RR (n+1) plots are accumulated around a circular shape. In contrast, plots of the mechanically ventilated group are accumulated around an elliptical shape.

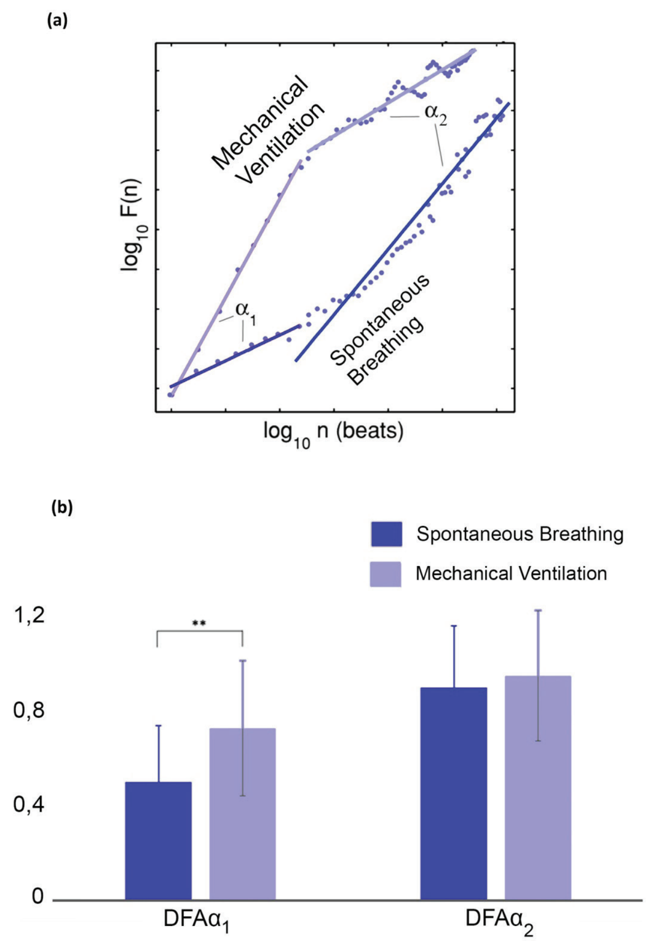

Figure 5 (a) Representative linear relationship between fluctuation function "logF(n)" and windows size "log10 n (beats)" (b) DFAα_1 (short-term) and DFAα_2 (long-term) scaling exponents in both spontaneous breathing and mechanically ventilated group. ** $p<0,01$.

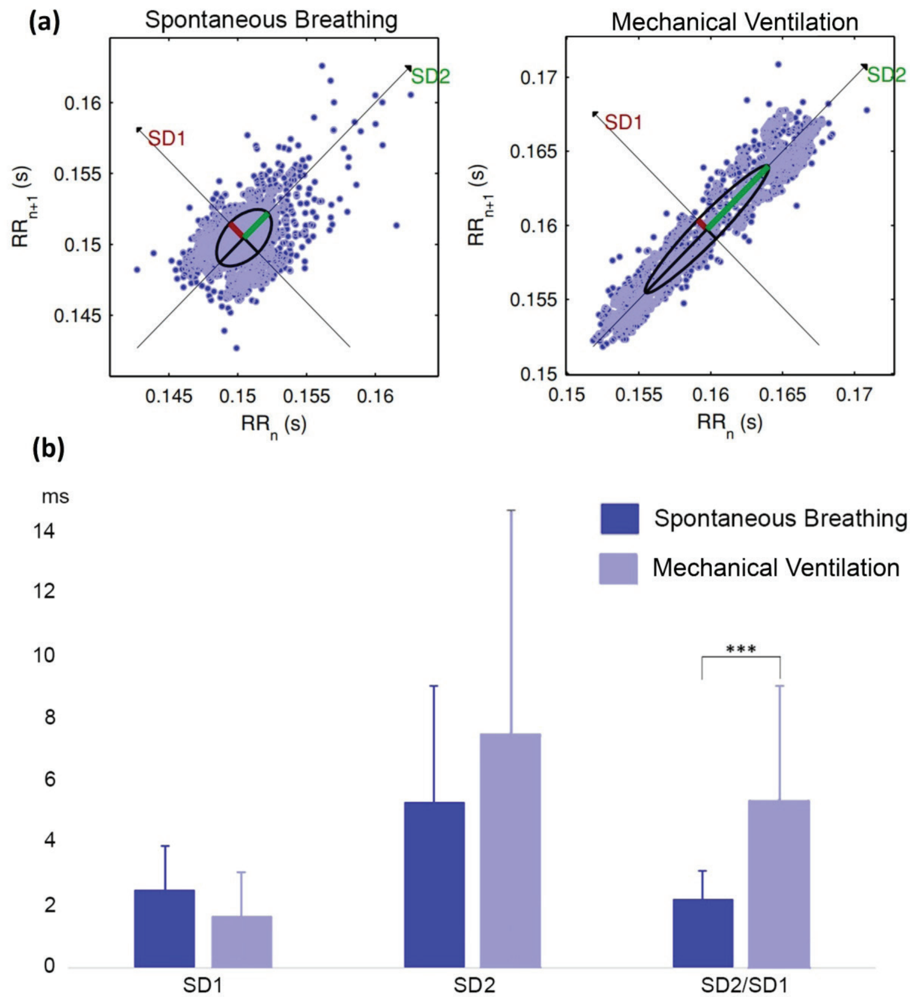

Figure 6 (a) Representative Poincaré Plot graphics of two animals from each group. (b). Poincaré Plot analysis results of both spontaneous breathing and mechanically ventilated animal groups. ***: p<0,001 compared to spontaneous breathing.

Figure 6b illustrates Poincaré plots representing the means of group values.

Calculated mean SD1 values were 2,51 ± 1,40 ms and 1,67 ± 1,40 ms and calculated mean SD2 values were 5,34 ± 3,72 ms and 7,50 ± 7,17 ms in spontaneous breathing and mechanically ventilated groups, respectively. Finally, mean SD2/SD1 ratio was 2,20 ± 0,93 in spontaneous breathing and 5,36 ± 3,70 in mechanically ventilated group.

According to these findings, while mean SD1 decreased, mean SD2 was found to be increased. Even though both changes were insignificant, the mean ratio of SD2/SD1 was found to be increased significantly in the mechanical ventilation group (p<0,001).

Entropy Analysis

In the entropy analysis, ApEn and SampEn were compared between the two groups (see, Figure 7.).

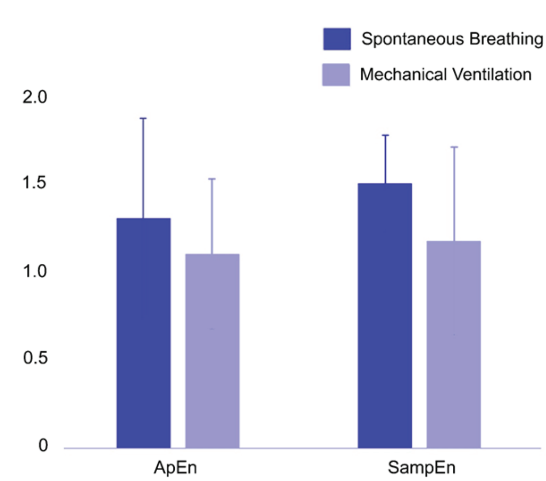

Figure 7 Mean ApEn and SampEn in both spontaneous breathing and mechanically ventilated group.

Calculated mean ApEn values were 1,31 ± 0,28 and 1,21 ± 0,34, and the calculated mean SampEn values were 1,51 ± 0,40 and 1,30 ± 0,46 in the spontaneous breathing and mechanically ventilated groups, respectively. Both of the mean ApEn and SampEn values have decreased insignificantly ($p>0.05$).

DISCUSSION

This is the first investigation to report significant changes in HRV in mice without any other alterations rather than ventilatory status. In the present study, we evaluated the effects of mechanical ventilation on the frequency domain and non-linear HRV analysis results under Na-Pentobarbital anesthesia in mice. (Xiuying, Abboud and Chapleau, 2002; Yan *et al.*, 2009) showed that activity of autonomic nerves including vagal efferents persists under Na-Pentobarbital anesthesia in mice (Xiuying, Abboud and Chapleau, 2002; Yan *et al.*, 2009). Thus, our discussion will be focused not on effects of Na-Pentobarbital anesthesia but on mechanical ventilation.

Our frequency domain HRV analysis results showed that mechanical ventilation decreased HF parameter significantly however did not change LF or VLF parameters. It has been suggested that VLF reflects thermoregulation, the renin-angiotensin-aldosterone system. And other slow changes which reflect on VLF parameter were found to be related to peripheral vascular system (Kitney, 1980). Whereas LF reflects changes in the sympathetic nervous system (Shaffer and Ginsberg, 2017). Frazier et al. did not observe any significant change in frequency domain parameters in 6 male mongrel canines (Frazier, Moser and Stone, 2001). (Borghi-Silva *et al.*, 2008) found that bi-level positive airway pressure (BiPAP) reduced HF parameter and did not change VLF and LF significantly in patients with chronic obstructive pulmonary disease (COPD) (39). (Bloomfield *et al.*, 2001) showed that HF parameter perished during breath-holding task (Bloomfield *et al.*, 2001). (Pagani *et al.*, 1984) found that controlled breathing increased HF parameter in healthy subjects (Pagani *et al.*, 1984) although Melo et al (2018) reported decreased HF parameter during controlled breathing (Melo *et al.*, 2018). In accordance with the literature, our findings indicate that respiratory status or breathing patterns may affect vagal efferents which in turn may alter sympathovagal balance. The altered sympathovagal balancemay be caused by the Hering-Breuer reflex which inhibits inspiratory muscle activity by decreasing vagal activity when the lungs inflate to a certain threshold (Bartlett and St. John, 1988). This reflex also leads to decreased HR caused by increased abdominal pressure and intrapleural pressure (Bartlett and St. John, 1988).

The Poincaré plot is a non-linear analysis method that reflects nature of the beat-to-beat intervals geometrically. Whereas SD1 is reported to be the analogue of HF parameter, SD2 considered as the analogue of LF parameter. Therefore SD2/SD1 ratio is comparable with LF/HF ratio (Guzik *et al.*, 2007; Shaffer and Ginsberg, 2017). In our model, there was no significant difference in SD1 and SD2 between spontaneous breathing and mechanically ventilated groups. Although, we observed that the distribution shape of the plots was different. The spontaneous breathing group's plot shape was more circular compared to mechanically ventilated group's plot shape. Our analysis also showed that the ratio of SD2/SD1 was increased significantly due to mechanical ventilation. The change of the plot shapes also correlates with this finding. Parallel to our results, Guzik et al. (2005) found that the increased respiratory rate caused a significant reduction of SD2/SD1 (Guzik *et al.*, 2005), which may mean that SD2/SD1 ratio is in fact sensitive to RSA. Brennan et al. (2001) stated that strong RSA usually occurs above the line of identification as a spur, indicating a rapid slowing of the heart rate. The presence of this trait makes the Poincaré plot a valuable tool in RSA assessment (Brennan, Palaniswami and Kamen, 2001). However, in our study the spur shaped differences occurring above the line of identification were not observed. We believe that this may be caused by the differences between analysis time periods or study design.

In our study, mechanical ventilation did not change ApEn and SampEn values when compared to spontaneous breathing group at our analysis conditions ($m=2$, $r=0,2$). Parallel to our findings, Gonçalves et al. (2008,2013) found that both ApEn and SampEnwere not affected by mechanical ventilation in rats (Gonçalves *et al.*, 2008, 2013). Weippert et al. (2015) found that ApEn, SampEn were strongly affected by metronome breathing (Weippert *et al.*, 2015), which may mean that both ApEn and SampEn are affected by changes in the respiration pattern but not mechanical ventilation.

Peng et al. (1995) noted in the initial article introducing the DFA algorithm that the DFAα exponent (box size: 4-16) is likely due to the physiological interbeat interval fluctuation. This fluctuation is dominated by the relatively smooth heartbeat oscillation associated with respiration on very short time scales (Peng *et al.*, 1995). Addition to this, results of the present study showed for the first time that DFAα_1 reflects not only changes of breathing frequency but also reversed respiratory physiology by mechanical ventilation. In accordance with this, DFAα_1 (box size:4-11) was increased in response to mechanical ventilation in our study. The only systematic research about the effects of breathing frequency on the short-term DFA exponent by Penttila et al. (2003), stated that the scaling exponent was increased by a reduction in respiration rate from 15 to 6 breaths per minute (Penttilä *et al.*, 2003). However, the authors offered no explanation for this finding. Weippert et al. (2015) found that metronome breathing increased DFAα_1 and decreased DFAα_2 in a study with 24 healthy individuals (Weippert *et al.*, 2015). Perakakis et al (2009) found reduced DFAα_1 when the breathing frequency of 14 healthy volunteers raised from 0.1Hz to 0.2Hz (Perakakis *et al.*, 2009). Our study confirmed the original argument by Peng et al. (1995) that periodic breathing oscillations are responsible for the crossover at scales close to the respiratory period (Peng *et al.*, 1995).

In our study we found a decreased HF parameter and an increased DFAα_1, which further support the notion that HRV is dominated by RSA at high frequencies, due to respiratory regulation pattern of vagal discharge to the heart (Denver, Reed and Porges, 2007; Grossman and Taylor, 2007); a very well-known mechanism. In addition, the time delays in sympathetic signalling pathways due to second messenger cAMP for the depolarization of pacemaker cells in the SA node, also strengthen the hypothesis that high-frequency HRV parameters such as HF parameter and DFAα_1, is driven by the parasympathetic system alone (Perakakis *et al.*, 2009).

On the other hand, there is another aspect of the increased DFAα_1 due to mechanical ventilation. As mentioned in the other sections, detrended fluctuation analysis deals with "non-periodical" and or "quasi-periodical" alternations of the beat-to-beat heart rate changes (Penzel *et al.*, 2003), which means DFA parameters are indicators of cardiac complexity (Peng *et al.*, 1995). Fractal complexity was increased solely by mechanical ventilation while irregularity remained unchanged according to ApEn and SampEn analysis results of our study.

CONCLUSION

The present study provides evidence that SD2/SD1 ratio, HF parameter and DFAα_1 are significantly affected by mechanical ventilation. When compared with the rest, DFAα_1 and HF parameter better reflect mechanical ventilation induced changes in vagal activity than the behavior of HR and other HRV indices. Moreover, we have also demonstrated that fractal complexity was increased solely by mechanical ventilation while irregularity remained unchanged. These changes might be the result of decreased vagal tone caused by activation of various reflex mechanisms triggered by mechanical ventilation. This novel results of HRV analysis are important considering increased usage of HRV techniques day by day in animal models and other medical practices. It may also be concluded that to correctly interpret short-term HRV scaling behavior, it is important to consider respiration and heart rate together.

LIMITATIONS

This study may have several limitations. As with any study, comparisons may be confounded by differences between groups other than mechanical ventilation. Nevertheless, given the selected 5-minute duration of the RR-interval data, the physiological significance of the HRV may be under-represented in our results and analyses. Some limitations have to be considered when comparing results of the present study with other (short-term) analyses. Different definitions of ranges for the frequency bands and DFA short and long-term correlations (box sizes) may contribute to the differences in obtained results when compared with other studies.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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