Articles in PresS. Am J Physiol Heart Circ Physiol (January 29, 2004). 10.1152/ajpheart.00655.2003

Individual differences in Respiratory Sinus Arrhythmia

By

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Running title: Individuality of Respiratory Sinus Arrhythmia

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ABSTRACT

To investigate the inter-individual differences in respiratory sinus arrhythmia (RSA), recordings of ventilation and electrocardiogram were obtained on 12 healthy subjects for five imposed breathing periods (T_{TOT}) surrounding each individual's spontaneous breathing period. In addition to the spectral analysis of the R-R interval signal at each breathing period, RSA characteristics were quantified by using a breath-by-breath analysis where a sinusoid was fitted to the changes in instantaneous heart rate in each breath. The amplitude and phase (or delay = phase x T_{TOT}) of this sinusoid were taken as the RSA characteristics for each breath. It was found that for each subject the RSA amplitude-T_{TOT} relationship was linear whereas the delay-T_{TOT} relationship was parabolic. However, the parameters of these relationships differed between individuals. Linear correlation between the slopes of RSA amplitude vs. T_{TOT} regression lines and (i) mean breathing period and (ii) mean R-R interval during spontaneous breathing, were calculated. Only the correlation coefficient with breathing period was significantly different from zero indicating that the longer the spontaneous breathing period the lesser the increase in RSA amplitude with increasing breathing period. Similarly, only the correlation coefficient between the curvature of RSA delay-T_{TOT} parabola and mean breathing period was significantly different from zero: the longer the spontaneous breathing period the larger the curvature of RSA delay. These results suggest that the changes in RSA characteristics induced by changing the breathing period may be explained partly by the spontaneous breathing period of each individual. Furthermore, a transfer function analysis performed on these data suggests inter individual differences in the autonomic modulation of the heart rate.

Key words: human, heart rate variability, autonomic control of heart rate, individuality of breathing pattern

INTRODUCTION

Respiratory sinus arrhythmia (RSA) is often considered to be a valid index of vagal control justified by the highly linear relationship between (i) vagal efferent activity and the magnitude of RSA in spontaneously breathing anesthetized dogs (18) and (ii) the R-R interval and RSA amplitude during progressive cholinergic blockade for a given subject (19, 24). These relationships are related to the cardiac vagal outflow and to the cardiac vagal tone respectively. RSA is also affected by other aspects of autonomic cardiac control such as the parasympathetic baroreflex (9, 6) and it has recently been shown that the cardiac sympathetic outflow may also modulate RSA (28).

In addition to this "multidimensional" aspect to parasympathetic cardiac control the between-individual differences in RSA increase the difficulty of RSA interpretation. In the latest committee report on heart rate variability, method and interpretation (3), this between-individual aspect is mentioned several times leading to the conclusion that 'caution needs to be exercised in interpreting RSA, especially for between-subjects comparisons'. For example, the relationship between RSA amplitude and pharmacologically defined vagal tone when investigated for a number of subjects appears to be less close than that found within a given subject (15, 20). The increase in RSA amplitude with increasing breathing period also varies amongst individuals (17, 4).

Given the importance of the respiratory modulation of human autonomic rhythms and the prepotency of breathing in generating respiratory frequency rhythms (1), the incidence of the spontaneous breathing rate is worth clarifying as there exist an individuality of the breathing pattern (26, 2) which has been reported in several physiological conditions (27, 12). We investigated the role of spontaneous breathing period in RSA changes induced by breathing period changes. To quantify RSA we used a breath-by-breath analysis where a sinusoid is fitted to the instantaneous heart rate for each breath (23). The amplitude and the

phase (or delay) of this sinusoid constitute the characteristics of RSA for that breath and mean values are calculated for each breathing period. We thus quantified RSA in healthy subjects at various breathing rates

Our hypothesis was that the rate of increase in RSA amplitude with respiratory period might be similar in all subjects, provided a comparable range of breathing rates is examined. We defined this comparable range by starting from the individual spontaneous respiratory frequency of each subject and by choosing breathing rates surrounding this spontaneous breathing rate. Our results show that, even under these conditions, difference in the rate of increase in RSA amplitude with the breathing period existed between individuals. Similarly, difference between individuals was found in the parameters of the parabola fitted to the delay-breathing period relationship. However, these differences (the rate of increase in RSA on one hand and the curvature of RSA on the other hand) were correlated with the spontaneous breathing period and not with the mean R-R interval.

These results suggest that in all individuals there are changes in RSA characteristics with T_{TOT}, but there are differences between individuals in the feature of these changes. These differences appear to be related to the spontaneous breathing period of the subjects.

In addition, this protocol gives the possibility of performing a transfer function analysis of RSA control system (25). Indeed, the plot of the normalized RSA (amplitude of RSA /V_T) versus T_{TOT} provides the 'gain' of the system at each T_{TOT} for each individual.

METHODS

Subjects. Twelve healthy volunteers between 18 and 28 years of age, seven of whom were male, participated in the study (mean ± SD - height: 173.5 ± 7.8 cm; weight: 64.7 ± 10.4 kg; age : mean 22.9 ± 3.1 years). Informed consent was obtained from all subjects. The experimental protocol was examined and approved by the Institutional Ethics Review Board.

Experimental protocol. Subjects were comfortably seated and wore a facemask on which a flow meter (Fleish head No.1) and a differential pressure transducer (163PC01D36, Micro Switch) were mounted. Leaks from around the mask were checked for, prior to initiating recording, using an infrared CO_2 analyzer (Engström Eliza/Eliza MC). End tidal CO_2 (FET_{CO_2}) was measured continuously using the same apparatus and an electrocardiographic trace (ECG) was obtained covering the whole of the recording period. For each subject, Six series of 5 to 10 minute recording were performed, the first corresponding to spontaneous breathing, and then for the following five randomly sorted sequences: at an imposed frequency fixed at the mean spontaneous respiratory frequency, and at the mean spontaneous respiratory frequency plus and minus 3 and 6 breaths/min. For subjects with a low spontaneous respiratory frequency the minus 3 and 6 recordings were replaced by minus 2 and 4. The highest breathing rate observed amongst our subjects was 17 breaths /min so recordings for that subject were performed for 11, 14, 17, 21 and 23 breaths /min whereas the lowest breathing rate observed was 7 breaths /min with recordings accordingly performed at 3, 5, 7, 9 and 11 breaths /min.

To impose the breathing rate, an auditory cue was used which signaled only for the inspiration to begin. Hence, the inspiratory/expiratory time ratio, as well as the tidal volume, was chosen by the subject. However, if $F_{ET}CO_2$ departed more than ± 0.4% from the control level, subjects were requested to change their tidal volume accordingly. In fact, in most cases, during imposed frequency breathing subjects are inclined to hyperventilate and thus, before recording was started, they were asked to decrease the tidal volume.

Data acquisition. The acquisition of the data was carried out on a Macintosh microcomputer equipped with an analog-digital interface card. Sampling rate was 256 Hz. In order to calculate the respiratory period (T) and tidal volume (V_T), and to study heart rate variability (HRV) and RSA, a breath-by-breath analysis of all recordings was performed. The ECG

signal was processed and the R-R interval series were extracted and displayed on the computer screen in order to verify that the signal exhibited no noticeable trend and to show up possible errors. Mean and standard deviation of the R-R intervals were calculated for each recording. R-R intervals were interpolated linearly at 0.25 sec intervals to obtain equidistant time samples, and spectral analysis was performed using a recording length of at least 1024 sample data points.

Data Analysis A Fourier Transform procedure was applied to obtain the low (LF: 0.04-0.15 Hz) and high (HF: 0.15-0.40 Hz) frequency components. For each recording, a restricted respiratory frequency component identified as the Respiratory Centered Frequency (RCF) component was also calculated, using the frequency range corresponding to ± 10% of the respiratory rate averaged over the entire recording (23). The power corresponding to the different frequency ranges was expressed as a percentage of the total spectral power minus that corresponding to the very low frequencies (0 - 0.04 Hz).

A more specific analysis of RSA was performed using a breath-by-breath HRV analysis (23). In order to quantify the extent of within-respiratory cycle HRV a sinusoid was fitted to the changes in instantaneous heart rate within the respiratory cycle. For each breath, the maximum value of the sinusoid is expressed as a percentage of the mean cardiac frequency calculated over that breath. This maximum value is used as a measure of RSA amplitude. The time elapsing between the beginning of the breath and the occurrence of this maximum value is expressed either in terms of the fraction of breath duration (phase) at which it occurs or in seconds (delay = phase x T). Average amplitude, phase and delay values over several breaths were then calculated for each recording. In addition, for each breath, a normalized RSA amplitude (RSA amplitude divided by the corresponding V_T) was calculated.

Statistical analyses. Values were expressed as mean ± SD. Mean values of the various variables between spontaneous and imposed breathing at the same rate were compared using paired t-test whereas SD's were compared using Wilcoxon paired-test.

To compare heart rate between different recordings for a given subject, mean R-R intervals at different imposed breathing frequencies were compared using an Anova analysis.

Linear correlation coefficients and regression lines were calculated for each subject from amplitude against breathing period plots using all the values available for this subject (250 to 300 breaths). In order to test the hypothesis of parallel regression line for all subjects, a linear regression line was also calculated using all the values of all subjects. The differences in slope between this common line slope and the individual slopes were calculated and the sum of the weighted differences was compared using a chi-square test with 11 degrees of freedom as it applies to 12 subjects.

Parabolic fit was calculated for each subject on delay versus breathing period plots. And also one parabolic shape was adjusted using all available values of all subjects. As in the case of the regression lines, to test the hypothesis of the existence of a common parabola, the sum of the weighted differences between the curvature parameter of the common parabolic shape and those of the individual parabolas were calculated again using a chi-square test.

For all tests, significance was set at $p<0.05$.

RESULTS

The values of T_{TOT} and R-R interval during spontaneous breathing are given in Table 1.

RSA for spontaneous and imposed breathing at the same frequency:

Figure 1 shows an example of two recordings on one subject when breathing spontaneously (left) and when breathing at an imposed frequency equal to the spontaneous breathing rate (right). Below are represented the instantaneous heart rate -delimited breath by breath- and the corresponding spectral analysis for the whole recording.

Comparison of the TTOT, VT, R-R interval and RSA analyses for spontaneous and imposed breathing at the same rate for all 12 subjects is illustrated in Figs 2 and 3. The *p*-values of the tests corresponding to the different variables for mean values and SD are given in the legend to the figures. In Fig. 2a is plotted the mean imposed breathing period against the spontaneous period for all subjects. Although, there was no significant difference between the two conditions as to mean value, the imposed breathing appears to be more regular as shown by a smaller SD. The tidal volume (Fig. 2b) was slightly, but significantly, higher during imposed breathing with however no difference in SD as regards the two conditions. Nor was any significant difference found in the inspiratory durations (TI) and TI/TTOT ratio with p=0.082 and p=0.133 respectively. Comparison of the R-R interval (Fig. 2c) as regards the two conditions showed no difference either in mean value or SD.

The power of the spectral components of the R-R interval signal was also compared for the two conditions: there was no significant difference in either the LF (p=0.422) or HF component (Fig. 3a), whereas the RCF component was significantly higher for the imposed frequency condition due to more regular breathing in this condition.

RSA amplitude and delay were also compared between the two conditions (Fig. 3 b and c) and there was no significant difference as regards either mean values or SDs.

RSA changes for imposed breathing frequencies:

Spectral analysis

Figure 4 shows the RCF and HF components of all the imposed breathing frequency recordings for the 12 subjects. As expected, the HF component falls for respiratory periods longer than 6.78 sec (0.15 Hz), whereas the RCF component exhibits a plateau at about 80% of the total power and this plateau is reached for values of TTOT of between 6 and 7 sec.

RSA amplitude

RSA amplitude increased with increasing TTOT. For each subject the coefficient of linear correlation between RSA amplitude and TTOT was calculated on all breaths recorded for that subject. All 12 correlation coefficients differed significantly from zero and their values ranged from 0.91 to 0.99. Individual regression lines were also calculated for each subject. These regression lines are represented in Fig. 5 and individual values of the slopes are given in Table 1. The hypothesis of a common slope applying for all subjects was then tested using a chi square test. The value of the test was $\chi^2 = 698$, and thus the hypothesis is rejected ($p<0.001$).

In order to investigate the relationship between these individual regression lines and the subject characteristics' individual slopes were plotted versus mean R-R interval (Fig. 6 right) and also mean TTOT (Fig. 6 left) calculated from the recording at spontaneous breathing. It can be seen that whereas no correlation was observed with R-R interval (*r=0.135, NS)*, the linear correlation with spontaneous TTOT was significantly different from zero (*r=0.648, p<0.03*). The slope of the latter regression line is negative indicating that the higher the spontaneous TTOT the lower the rate of increase of RSA amplitude with respiratory period.

RSA delay

The delay increased with TTOT up to a certain value and then decreased. For each subject a parabolic fit was found. The coefficients of multiple correlation were significantly different from zero for all subjects and their value varied from 0.93 to 0.99 among subjects. These parabolas are represented in Fig.7. However, the parabola appeared to differ between individuals as it can be seen in Table 1, where are gathered the curvature and the coordinates of the maximum of individual parabola. The curvature ($2a$) of a parabola (ax^2+bx+c) is often used to characterize this parabola because it is independent of the origin. Applying a chi square test to explore the hypothesis of a parabola common to all subject lead to a rejection of the hypothesis for all the parabola curvature ($\chi^2 = 93.5$, $p<0.01$).

As above, we investigated the relationship between these parabola and the individual characteristics. We plotted the curvature versus mean R-R interval (Fig. 8 right) and mean TTOT (Fig. 8 left) calculated from spontaneous breathing. Only the correlation coefficient (*r=0.660, p<0.03*) between curvature and spontaneous TTOT was significantly different from zero indicating that greater curvature is associated with longer spontaneous TTOT.

RSA transfer function analysis

The normalized RSA amplitude (RSA amplitude/VT ratio) was plotted versus TTOT for each subject. Correlation coefficients were calculated for each subject. All coefficients were significantly different from zero and individual linear regression lines were calculated (Fig 9).

DISCUSSION

The main findings of this work were that (i) there exist differences between individuals in the RSA characteristics changes with TTOT and (ii) these differences may be – at least partly - explained by the differences in the spontaneous breathing period between subjects. Furthermore, inter individual differences in the heart rate modulation system are suggested by the results of a transfer function analysis performed on these data.

Spontaneous vs. imposed breathing

Prior to studying RSA corresponding to different imposed breathing periods, we felt that the RSA for spontaneous and imposed breathing at the same rate should be compared in order to ascertain that the respiratory period was the main explicative factor and thus to justify the study being carried out with imposed breathing rates.

In humans, volitional breathing arises from a cortico-motor excitation of the diaphragm, which may act, directly on the phrenic motor nucleus via the cortico-spinal tract ‘bypassing’ brain stem respiratory centers; alternatively, or in addition, this may be achieved indirectly via the respiratory centers and bulbo-spinal paths. There is no clear evidence for

these alternatives, although several studies suggest that the cortico-motor excitation of the diaphragm does not transit via the medullary respiratory centers (7). The observation of no difference in RSA between spontaneous and imposed breathing at the same rate does not provide indication in favor of either alternative. It merely suggests that for each individual breathing rate per se has an incidence on the characteristics of RSA. Heart rate variability and RSA, spontaneous or imposed, at the same breathing rate have been studied by several authors (17, 11, 16, 21, 22). In these studies, no difference was found in either mean heart rate or HF amplitude between spontaneous and paced, or metronomic, breathing. Our results in agreement with these observations lead to the conclusion that the voluntary control of breathing does not enhance vagal tone or alter the vagal modulation of heart rate.

The experimental protocol

We chose to impose breathing frequencies starting off from the spontaneous breathing rate of each subject. This was done because of the great variability in resting breathing rate among subjects (8) and also because this breathing rate appears to be an individual characteristic (2). Thus the recordings were not performed over the same range of breathing frequency for all twelve subjects and also the width of the frequency range used varied with the individual subject. This can be seen clearly in Fig. 5 where the extremities of a regression line show the extreme values of respiratory period obtained for the subject concerned. These differences may be considered as a bias in the experimentation but on the other hand it should be pointed out that a frequency of 12 breaths/min represents an increase in breathing rate for some subjects and a decrease for others and this may influence the regulatory mechanisms brought into play. In this study, for each subject, starting from a breathing rate corresponding to the spontaneous rate two higher and two lower rates were imposed, thus surrounding the spontaneous rate.

R-R interval: mean value and spectrum for different breathing period

Changing the respiratory period did not change the heart rate. This observation is in accordance with several authors (17, 4, 1) and, as suggested by Brown *et al.* (4) different within-subject breathing frequencies and depths distribute vagal firing within the respiratory cycle but do not alter the level of vagal outflow. Kollai and Mizsei (20) found that the mean heart period changed in response to slow breathing and that the nature of the change (increase, decrease or no change) varied with the individual. Differences in the experimental protocol and in the range of periods explored in each subject may explain this divergence.

The percentage of the power spectrum occurring in the HF and RCF bands of the instantaneous cardiac rate signal are represented in figure 4. It can be seen that, as expected, the power in the HF band is greater than power in the RCF band up to the period (6.7 sec) corresponding to 0.15 Hz and that beyond this there is a noticeable difference between the two plots: the values of HF fall whereas a plateau is reached for RCF which appears to be at around 80% of the total power spectrum. It should be noted that if the HF band is not suitable as a means of describing the power corresponding to breathing at low breathing rates, the RCF band has the disadvantage of varying in width with breathing rate.

Nevertheless, as the total surface of the power spectrum represents the variance of the analyzed signal, the percentage of the power in a given band – here RCF – may be considered to be the variance associated with this frequency range. Thus, the fact that the power in the RCF band reaches a plateau suggests that the extent of the heart rate variability dependent on breathing rate does not exceed 80 % of the total variability.

RSA Amplitude

All subjects exhibited an increase in RSA amplitude with increasing respiratory period at least in the explored range of periods. This observation is in accordance with the findings of Hirsch and Bishop (17) who estimated RSA amplitude as the difference between the lowest

and highest instantaneous heart rates in each breath and also those of other authors who used spectral analysis (10).

Our initial hypothesis, which was that parallel regression lines could be found, representing the RSA amplitude versus breathing period relationship for all subjects, was rejected by the statistical test which indicated inter-individual differences in this relationship. Similar results have been reported by Hirsch and Bishop (17) who plotted RSA amplitude vs. breathing rate for each subject on a log-log scale. They found a constant RSA for low breathing rates, below 3 to 7 breaths /min and then a decreasing relationship, the slope of which was expressed in decibels per decade and which defined the system roll-off. The origin of the decreasing relationship – low frequency intercept- varied amongst subjects as did the roll-off i.e. the slope of the log RSA-log breathing frequency plot.

In the same way as for the roll-off, the slope of RSA amplitude-breathing period relationship exhibits inter-individual differences and this slope was not related to individual's heart rate but correlated to their spontaneous breathing period. This slope, which represents the rate of change of RSA amplitude with TTOT, may be considered to be a measure of the responsiveness of the heart rate modulation mechanism to changes in respiratory period. The significant negative correlation with the spontaneous T_{TOT} suggests that subjects with a low spontaneous breathing period (high rate) will be more responsive i.e. their RSA amplitude will increase more with increasing period than for subjects with long breathing periods.

This responsiveness is somewhat different from the one defined by Kollai and Miszei (20). They defined an individual RSA responsiveness expressed as the ratio $\Delta HP/\Delta RSA$ where ΔHP is the change in heart period with increasing respiratory period. Because 3 types of ΔHP were found: individuals with (A) increasing, (Z) decreasing and (A/Z) unchanged, the slope of ΔHP - ΔRSA plot was respectively positive, negative or close to zero. This ratio was found to be correlated to parasympathetic control (PC) defined as the changes in heart period after complete parasympathetic blockade by the administration of atropine. Although there

was a continuum of distribution of subjects along the ΔHP/ΔRSA against PC regression line, the A type were mainly distributed on the left hand side of the graph (i.e. high responsiveness associated with low PC) and the Z type, mainly at the right hand end of the regression line. Kollai and Miszei (20) concluded that the inter-individual differences in RSA have their origin mainly in the differences in parasympathetic control, although they found that introducing respiratory characteristics (T_{TOT} and V_T) improved the degree of RSA – PC correlation.

RSA Delay

The delay is the time elapsing in each breath between the onset of inspiration and the reaching of the maximal value of the sinusoid fitted to the instantaneous heart rate. We chose the delay (Delay = Phase x T_{TOT}) rather than the phase because of its time dimension, which might make the interpretation of the results easier. This variable differs somewhat from the phase angle between the P-P interval and respiration reported by Eckberg (10), who measured the phase angle between the onset of inspiration and the heart period shortening. Although both Eckberg's study and the present one are concerned with the time lapse between inspiratory onset and heart rate acceleration, for Eckberg, the phase was defined by the start of the acceleration whereas in this study the delay was defined by the moment of the maximum acceleration. Nevertheless, in both studies a change occurred at periods in the range of 8 to 10 sec. Indeed, Eckberg (10), reported that at breathing intervals of 8 and 10 sec, P-P shortening began before the onset of inspiration, so that the polarity of the phase changed from positive to negative as the breathing period increased. In our study, the delay-T_{TOT} relationship is parabolic and for 8 out of 12 subject the maximum occurred in the 8 to 10 sec range (Table 1)

Our hypothesis was that of the existence of a common parabolic shape representing the RSA delay – T_{TOT} relationship for all subjects. This was based on the assumption that the time taken to develop the inspiratory parasympathetic inhibition on the heart and reach the maximum heart rate acceleration would be similar amongst subjects. Given our protocol, we

expected a parabolic fit only for those subjects with long TTOT. Our results rejected this hypothesis and as can be seen in Fig. 7, even for those subjects such as subject 1 and 2 with short spontaneous TTOT, a parabolic relationship exists between the delay and TTOT and the delay is reached earlier than at a TTOT value of 8 seconds. The curvature as also the other parameters of the parabolas were found to be related to the spontaneous TTOT indicating the influence of the latter on changes in RSA brought about by changing the breathing period.

One possible interpretation of these results would be that the pattern of the modulatory mechanism is affected - or even determined - by the spontaneous breathing period so that for subjects with a long spontaneous TTOT the modulation will be initiated either later in the breathing period or much more gradually than for those subjects with short a spontaneous breathing period.

RSA Transfer function analysis

In this study it can be assumed that in each series of recordings on one subject, the only variable that is changed is the breathing rate. Thus, the breathing rate changes may be considered as an input to the heart rate modulation system the output of which is RSA. As any change in the breathing rate is associated with changes in VT to compare the gain of the heart rate modulation system within- and between subjects the amplitude of RSA of each breath has to be normalized i.e. divided by its VT.

Figure 9 shows that the change in the gain of the system varies among individuals; in some subjects (#7, #9, #10) there are little changes, in contrast to some others (#1, #3, #12). Furthermore, over a fixed respiratory period range there are between individual differences in the gain of the system. This indicates that these differences are not due to the different amount of the input but rather to the characteristics of the system.

Thus, if one subject's line is "higher" than another subject's line, then the former subject has greater gain. If one subject's line intersects with another subject's line, then the former subject

has greater gain over a particular range of respiratory period and lesser gain over the remaining respiratory range.

Transfer function analyses have been used in physiological (25) and pathological (13) conditions where the gain of the system was quantified and considered as being a measure of the autonomic tone.

Individuality of RSA?

These results in addition to several other observations (15, 20, 4) on inter-individual differences in RSA changes suggest that there may exist an individuality of RSA. However, the existence of individuality implies not only differences between individuals but also reproducibility for a given subject. Little data is available on the reproducibility of RSA at a given period. Grossman *et al.* (14) and Grossman and Kollai (15) showed that behavioral tasks known to influence cardiac vagal tone produce closely corresponding within-subject changes in mean R-R interval and RSA when the respiratory parameters are controlled. The comparison of spontaneous and metronomic breathing as in this study and several other studies suggests that there was no difference in RSA amplitude between these two conditions even, in head up tilt and low body negative pressure situations as reported by Patwardhan *et al.* (22). For respiratory periods other than spontaneous, the RSA amplitude corresponding to an increased respiratory period induced by the addition of resistive load was similar to the RSA amplitude for the same imposed respiratory period (5).

In conclusion, in addition to the differences in RSA between individuals, there exist inter individual differences in the RSA control system response to changes in TTOT dependent on 1) the spontaneous breathing period and 2) on the strength of autonomic tone.

Therefore, these results suggests that, in addition to the influence of respiratory characteristics on the gating of sympathetic and vagal motoneurons responsiveness, the individual breathing rate may play a role in the build up of the parasympathetic control of heart rate.

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Acknowledgements

The authors wish to thank the subjects who participated in this study. We are grateful to Pierre Baconnier and Abdelkebir Sabil for valuable discussion on this manuscript and to Angelique Brouta for her technical assistance.

Figure legends:

Figure 1: Recording of spontaneous and imposed frequency breathing at the same rate. Above the ECG, 1/R-R interval ratio (instantaneous heart rate) is represented with arrows indicating each R wave. Below is the instantaneous heart rate for the whole recording and the resulting Fourier analysis spectrum with the delimitation of the VLF, LF and HF frequency bands. The central gray arrow is the mean breathing frequency for the recording and two gray arrows indicating ± 10% delimit the RCF band.

Figure 2: Comparison of T_{TOT}, V_T, and R-R interval for spontaneous and imposed breathing at the same frequency. The plot represents mean value and SD for imposed frequency against spontaneous breathing for all 12 subjects.

The mean values and SDs were compared between the two conditions. As regards the respiratory period (T_{TOT}), mean values (*p=0.947*) and SDs (*p=0.054*) were not significantly different. For the tidal volume (V_T), the mean value was significantly higher during imposed breathing (*p=0.037*) whereas SD was not significantly different (*p=0.057*). For the R-R interval, neither the mean value (*p=0.576*) nor the SD (*p=0.268*) differed significantly between the two conditions.

Figure 3: Comparison of HRV (power spectrum components) and RSA characteristics (amplitude and delay) for spontaneous and imposed breathing at the same frequency. The mean values and SDs (apart those for HF and RCF) for imposed frequency against spontaneous breathing condition are plotted for all 12 subjects.

The power in the HF band was not significantly different between the two conditions (*p=0.463*), whereas that in the RCF band was significantly higher (*p=0.028*) for imposed breathing.

There was no significant difference neither in Amplitude (mean value, *p=0.196* and SD, *p=0.625*) nor in Delay (mean value, *p=0.062* and SD, *p=0.348*) between the two conditions.

Figure 4: The power at HF and RCF for all the recordings and for all subjects.

The power is expressed as percentage of the total power minus that at VLF (very low frequencies <0.04 Hz).

Figure 5 – Amplitude - TTOT regression lines for all subjects.
The regression lines were obtained over all the breaths recorded for each subject. The extremities of each regression line correspond to the lowest and highest values recorded for that subject.

Figure 6 – Plot of RSA amplitude- TTOT regression line slopes against mean values of R-R interval (right) and TTOT (left) obtained from the spontaneous breathing recording.

Figure 7– Delay - TTOT relationship for all subjects.
The parabolic fit was calculated for all the breaths recorded for each subject. For each parabola the extremities correspond to the lowest and highest values recorded for that subject.

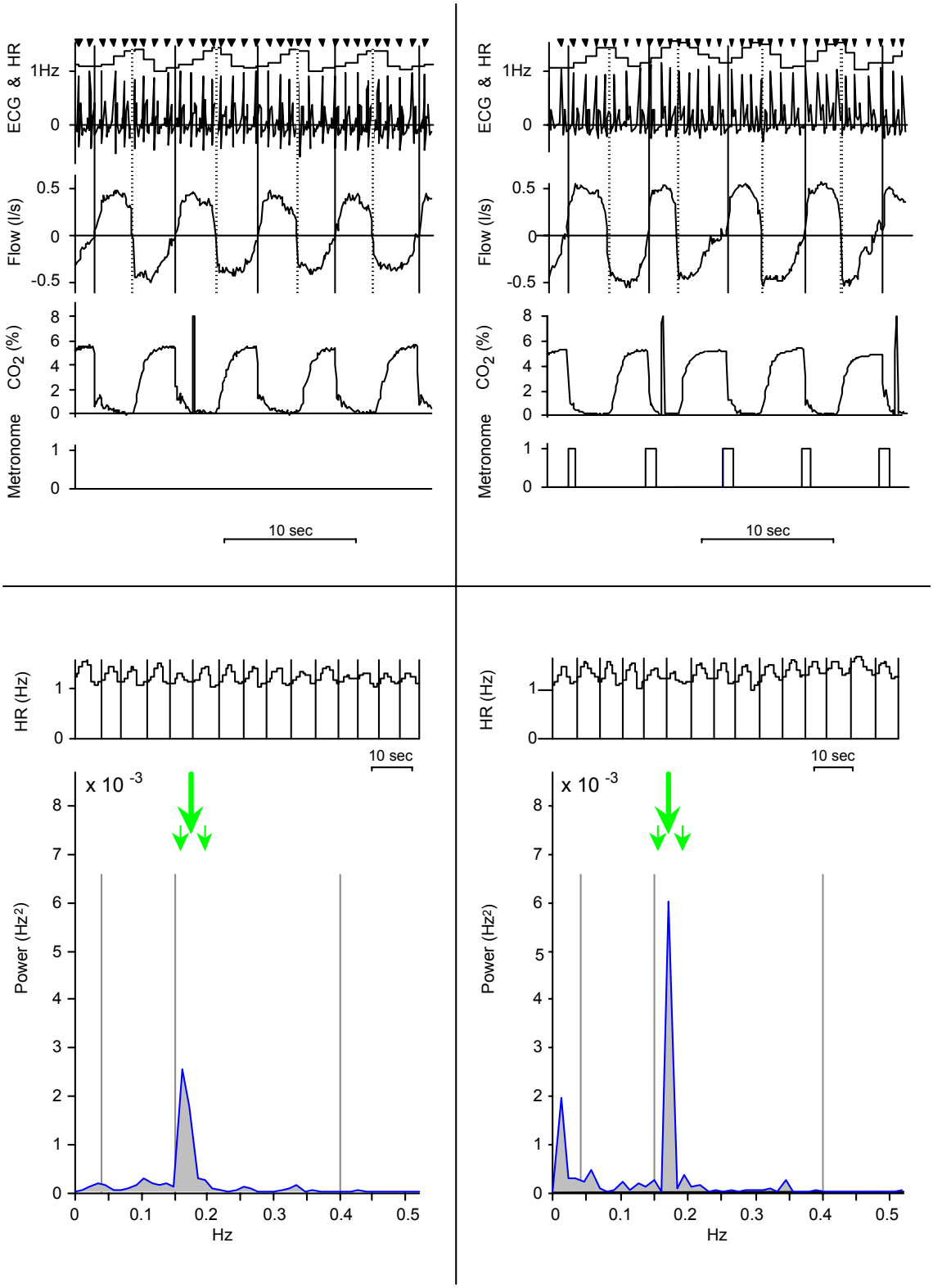
Figure 8 – Plot of curvature of the parabola plotted against the mean values of R-R interval and TTOT obtained from the spontaneous breathing recording. The curvature ($2a$) of a parabola (ax^2+bx+c) is often used to characterize this parabola because it is independent of the origin.

Figure 9 – Normalized Amplitude - TTOT regression lines for all subjects.
The regression lines were obtained over all the breaths recorded for each subject. The extremities of each regression line correspond to the lowest and highest values recorded for that subject.

Table 1 – Parameters of Amplitude = f (TTOT) and Delay = f (TTOT) relationships

Subjects	Spontaneous Breathing		Line fitting Amplitude = f (TTOT)	Parabola fitting Delay = f (TTOT)		
	TTOT (sec)	R-R interval (sec)	Slope	Curvature	Coordinates of the max	
					TTOT (sec)	Delay (sec)
1	3.3	0.799	0.041	-0.119	5.2	1.8
2	7.4	0.818	0.013	-0.027	8.7	2.5
3	5.3	0.617	0.022	-0.036	9.1	3.2
4	8.2	0.766	0.016	-0.041	8.4	3.3
5	7.5	0.780	0.017	-0.038	9.6	3.6
6	5.6	0.823	0.010	-0.041	8.5	2.9
7	4.1	0.886	0.015	-0.037	9.7	3.0
8	3.8	0.733	0.017	-0.048	8.5	3.2
9	4.4	0.834	0.021	-0.083	6.0	2.2
10	8.3	0.829	0.007	-0.017	13.2	3.7
11	6.5	0.923	0.009	-0.034	11.6	3.5
12	4.2	0.918	0.029	-0.050	8.5	2.9

The curvature ($2a$) of a parabola (ax^2+bx+c) is often used to characterize this parabola because it is independent of the origin.



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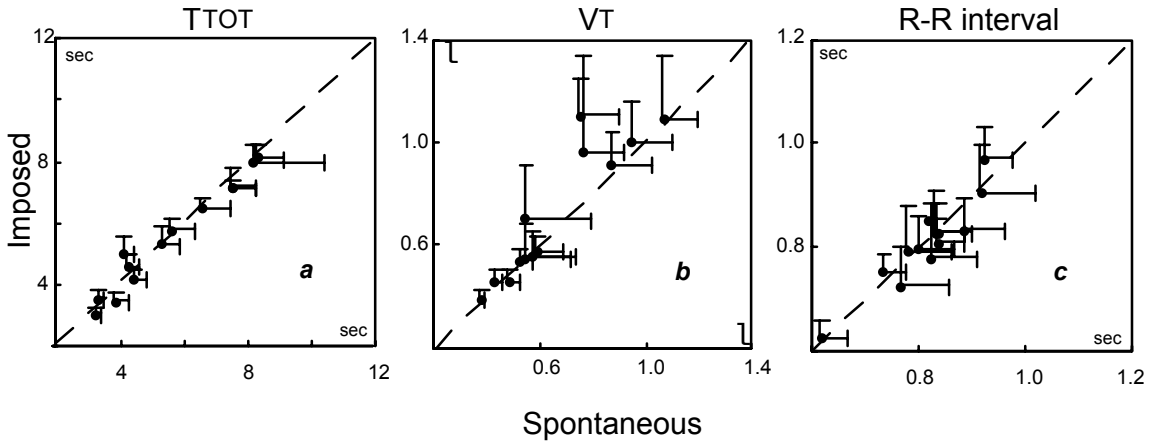
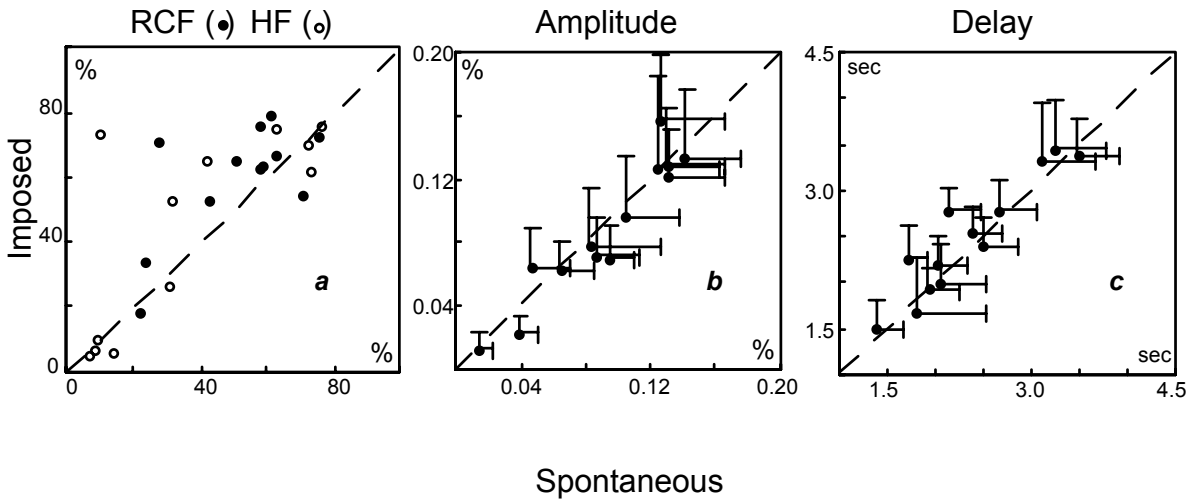


Figure 2: Comparison of TTOT, VT, and R-R interval for spontaneous and imposed breathing at the same frequency. The plot represents mean value and SD for imposed frequency against spontaneous breathing for all 12 subjects.

The mean values and SDs were compared between the two conditions. As regards the respiratory period (TTOT), mean values (*p=0.947*) and SDs (*p=0.054*) were not significantly different. For the tidal volume (VT), the mean value was significantly higher during imposed breathing (*p=0.037*) whereas SD was not significantly different (*p=0.057*). For the R-R interval, neither the mean value (*p=0.576*) nor the SD (*p=0.268*) differed significantly between the two conditions.



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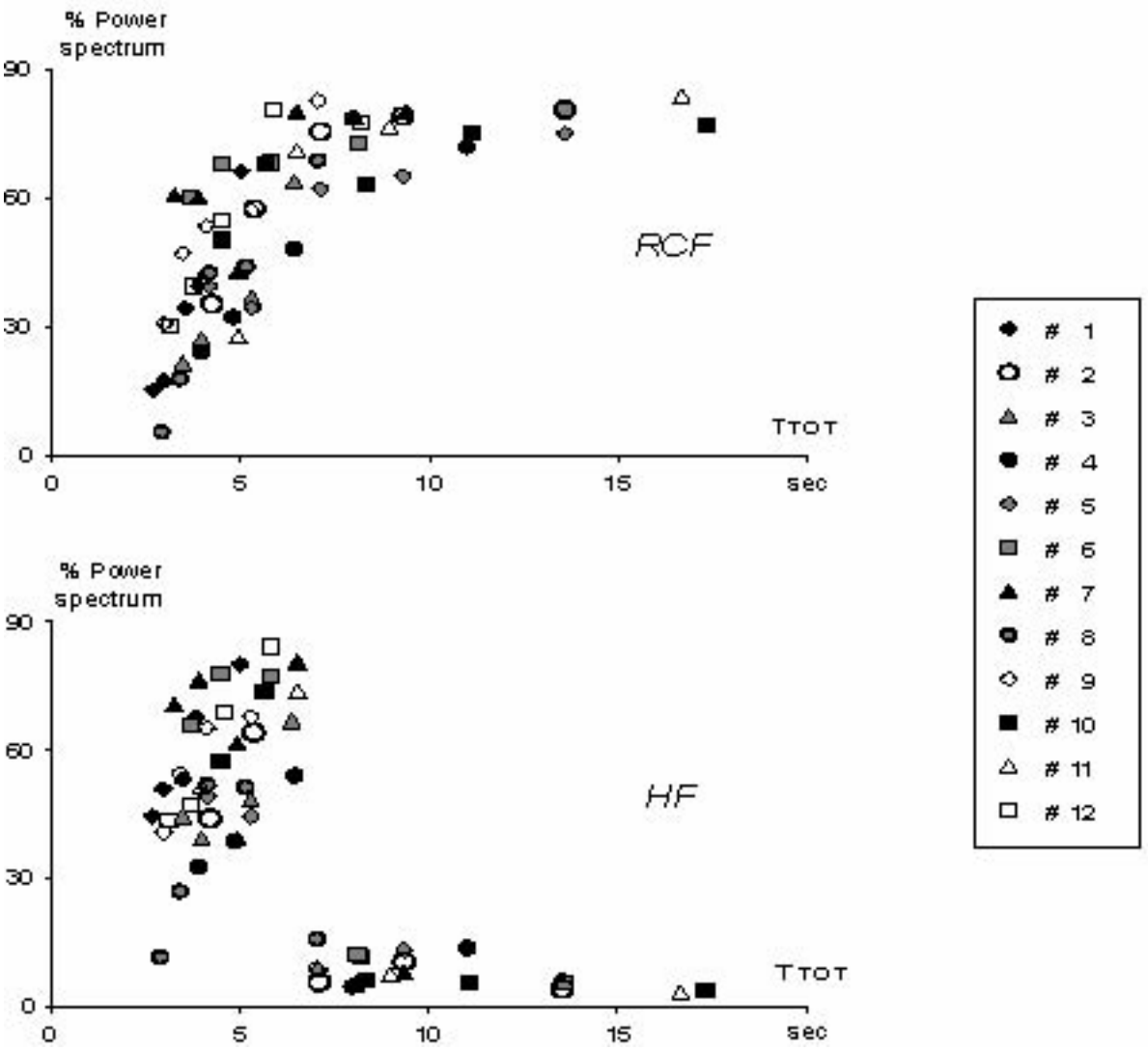

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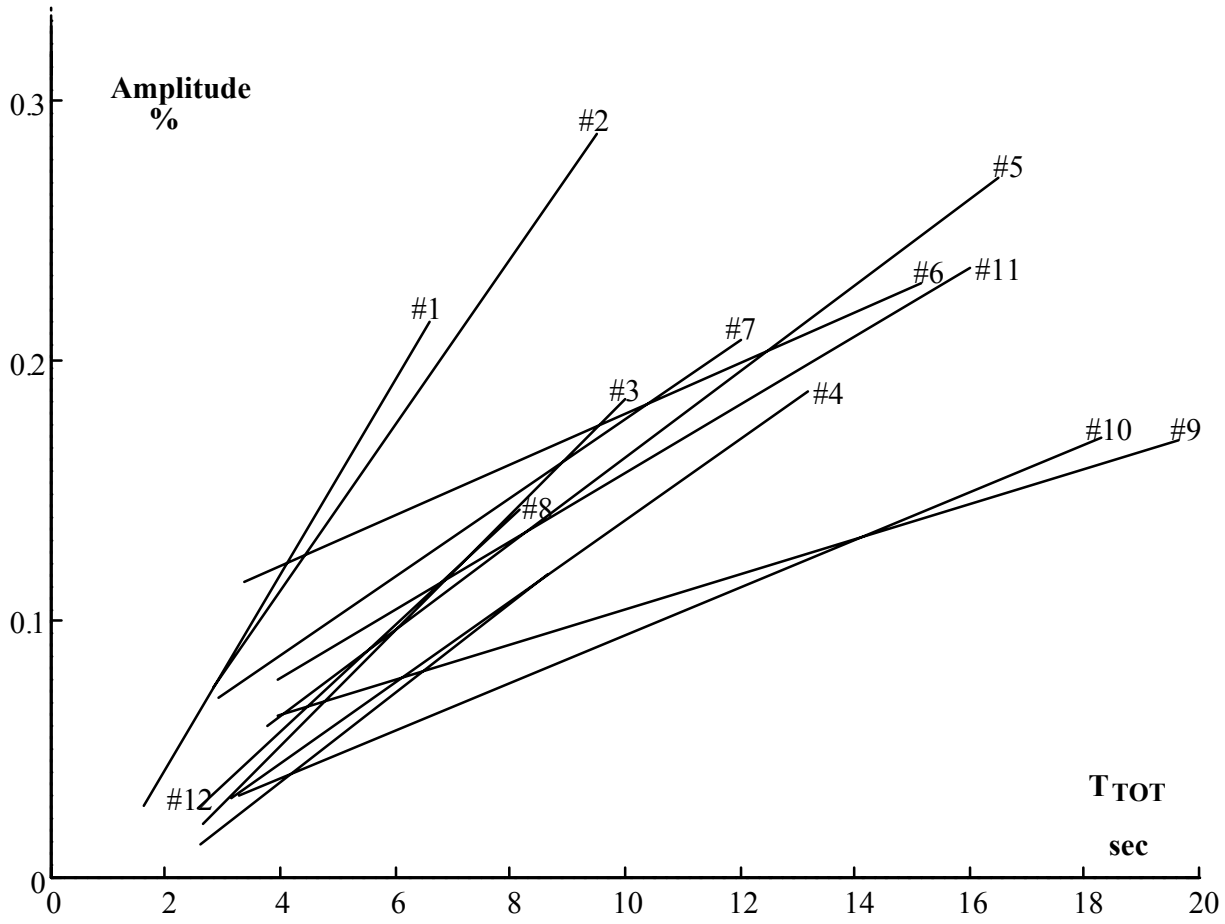


Figure 5 – Amplitude - TTOT regression lines for all subjects.

The regression lines were obtained over all the breaths recorded for each subject. The extremities of each regression line correspond to the lowest and highest values recorded for that subject.

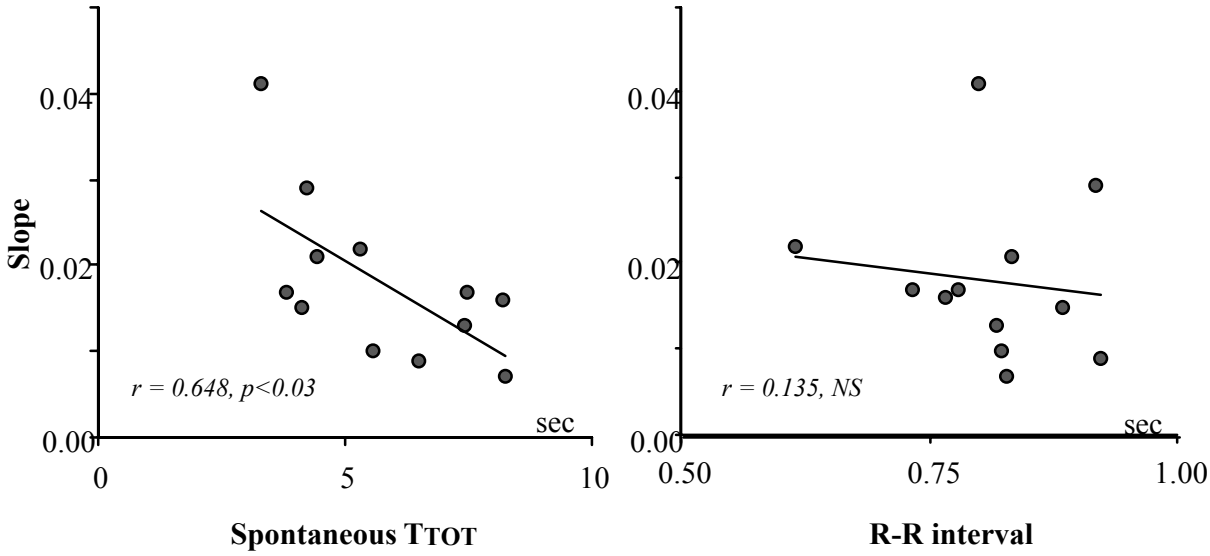


Figure 6 – Plot of regression line slopes against mean values of (a) R-R interval and (b) TTOT obtained from the spontaneous breathing recording.

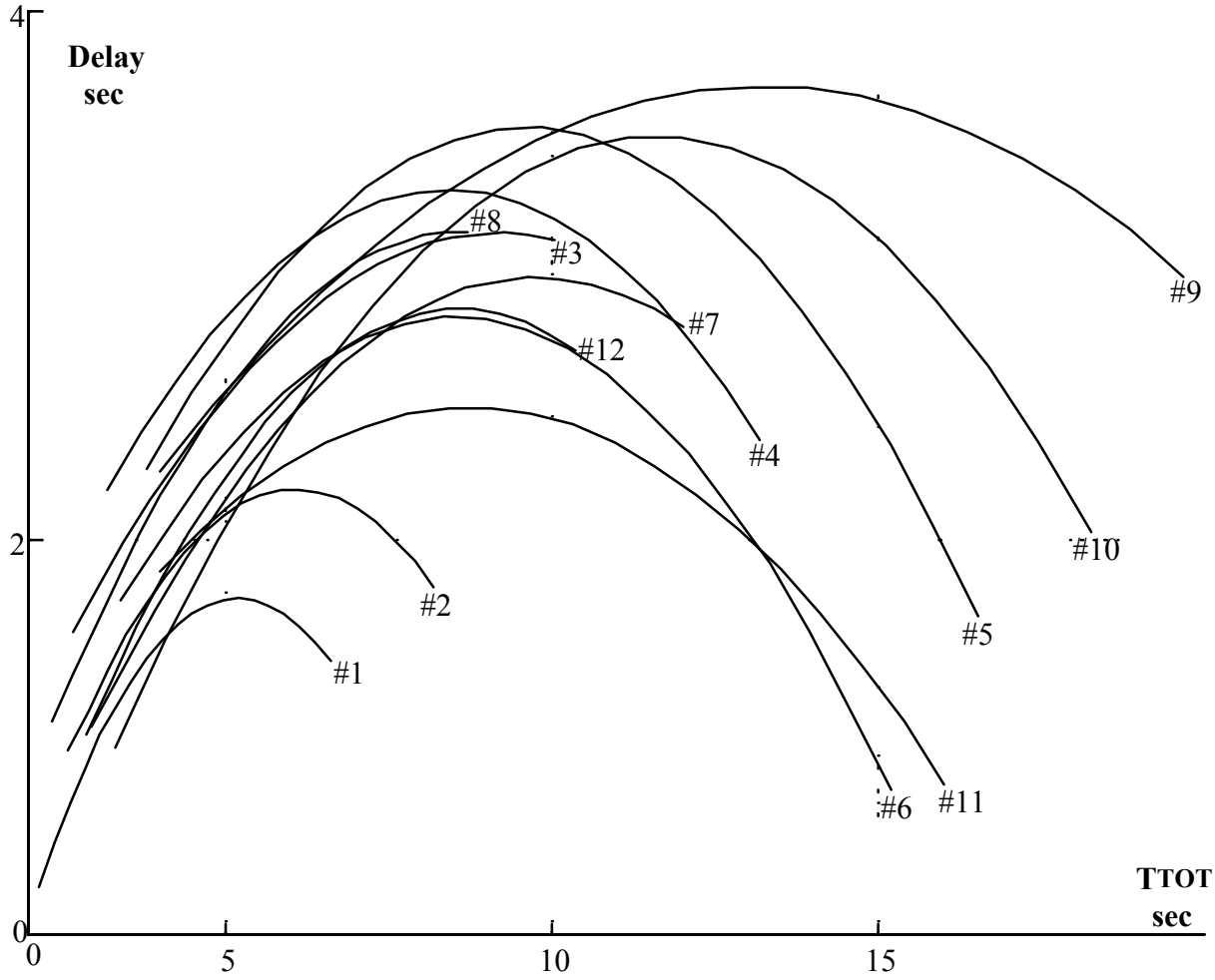
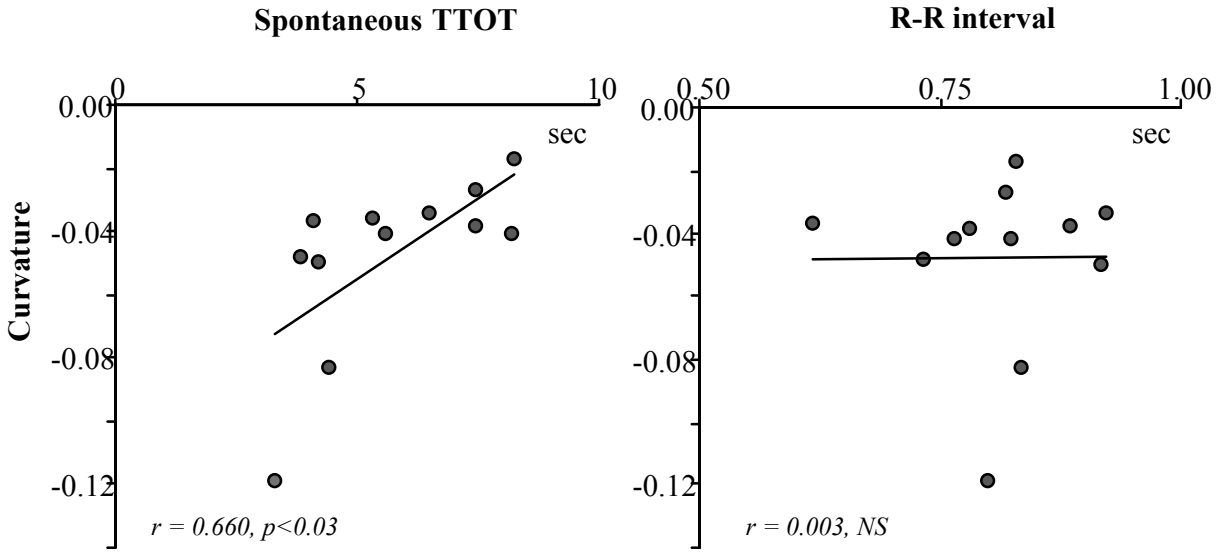


Figure 7– Delay - TTOT relationship for all subjects.

The parabolic fit was calculated for all the breaths recorded for each subject. For each parabola the extremities correspond to the lowest and highest values recorded for that subject.



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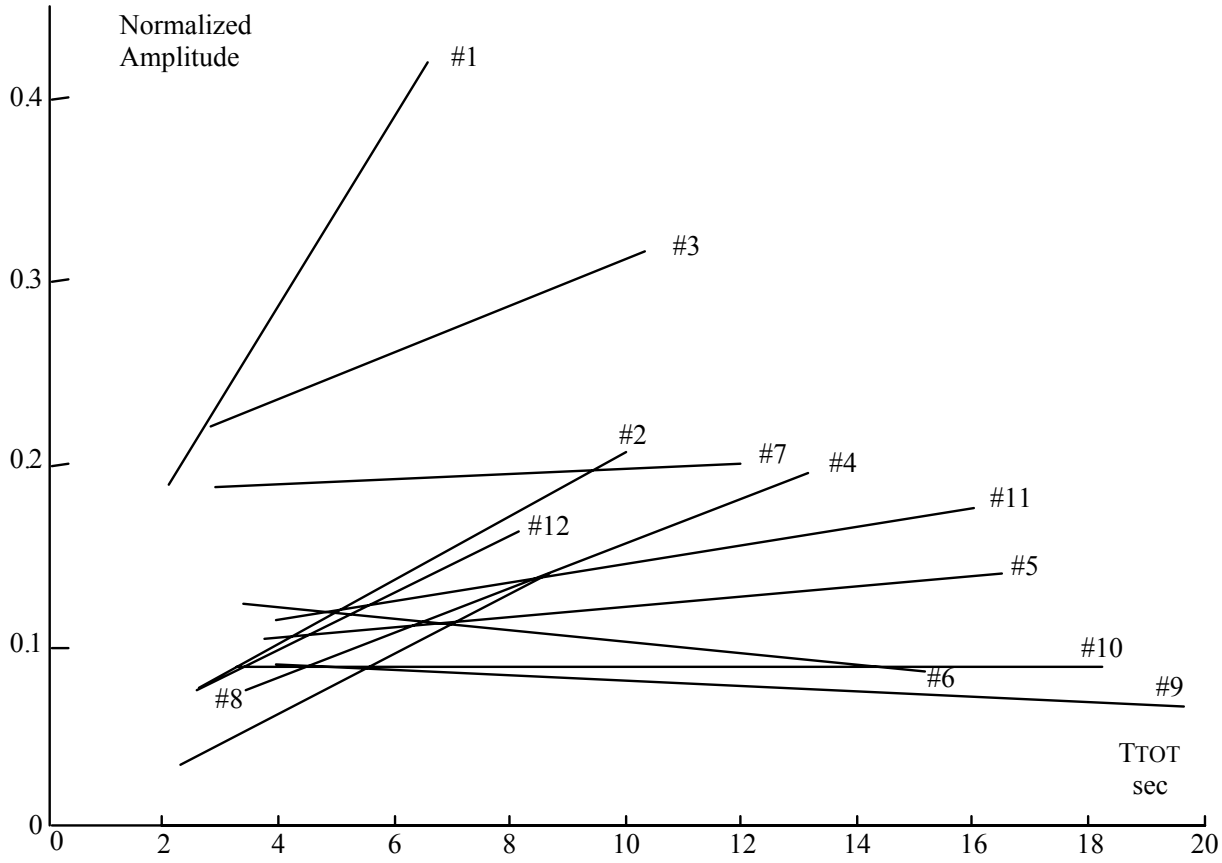

Figure 9 – Normalized Amplitude - TTOT regression lines for all subjects.
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