Human cardiovascular baroreflex regulation: different methods of measurement

Ph.D. Thesis

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List of publications

I. Zöllei, É., Gingl, Z., Kardos, A., Rudas, L.:
Comparison between different non-invasive indices of baroreflex gain.
Cardiol Hung 1999;28:269-272.

II. Zöllei, É., Paprika, D., Rudas, L.:
Measures of cardiovascular autonomic regulation derived from spontaneous methods and the Valsalva manoeuvre.
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III. Zöllei, É., Paprika, D., Makra, P., Vezendi, K., Rudas, L.:
Human autonomic responses to blood donation.
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IV. Zöllei, É., Csillik, A., Rabi, S., Gingl, Z., Rudas, L.:
Respiratory effects on the reproducibility of cardiovascular autonomic parameters.
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1. Introduction

1.1. Baroreflex regulation – basic physiology and clinical importance

The rhythmic fluctuation of heart rate and blood pressure is a fundamental characteristic of the cardiovascular system. The fluctuation of heart rate is influenced by several mechanisms, from which the respiratory sinus arrhythmia is well-known for a long time. The other main determinant of heart rate variability is blood pressure. Though we usually look at this mechanism assuming that arterial pressure change is the primary event, we know that heart rate itself has a feedback influence on blood pressure (Kardos et al., 1995). Modern physiology acknowledges cardiovascular autonomic regulation as a chain of several reflex mechanisms. From these, arterial and cardiopulmonary baroreflexes play the most important role in maintaining adequate circulation, e.g. in ensuring optimal arterial pressure and heart rate, and cardiac output. Arterial baroreflexes originate from the baroreceptor areas at the carotid sinuses and aorta. The stimulus for these receptor areas is the deformation of the vessel wall. The stretch induced by blood pressure changes initiate the reflexes, which exert its effect on heart rate (vagal efferents) and also on blood pressure (sympathetic efferents), though it is an oversimplification of the interrelation of vagal and sympathetic mechanisms. We also should not forget that events can have different, even opposing effects on carotic, aortic and cardiopulmonary baroreceptors, complicating interpretation even more.

In human beings we cannot directly measure the activity of the baroreceptors, neither the activity of the central nervous system. However, we can describe the variability of the heart rate and blood pressure (traditionally by time and frequency domain parameters), and also we are able to quantify the rapid (vagal) changes in RR interval in relation to blood pressure changes, the so-called cardiac vagal baroreflex gain (BRS). Sympathetic efferent responses are also assessable as the muscle sympathetic nerve activity (MSNA).

In the last decade we could observe a growing clinical interest in cardiovascular autonomic abnormalities. The reason is that several cardiovascular and neurological conditions are associated with autonomic dysfunction (Parkinson disease, diabetes mellitus, heart failure, post-myocardial infarction states). In some of them even the impaired autonomic regulation is considered as an important prognostic factor. It is thought to have great impact

on the risk of sudden cardiac death after myocardial infarction or heart failure (LaRovere et al., 1998; Mortara et al., 1997). Sympathetic activity is regarded as having arrhythmia-inducing, parasympathetic activity as having protective effects against life-threatening ventricular arrhythmias. Certain therapeutic measures, like certain drugs, physical exercise may exert beneficial effect through their action on autonomic regulation.

1.2.The different methods for baroreflex gain estimation

The assessment of cardiac vagal baroreflex gain as an index of baroreflex function is based on the quantification of RR interval (RRI) changes related to blood pressure changes. The two traditionally used techniques are the pharmacological and the neck chamber methods. The former **(Oxford method)** consists of blood pressure manipulations using vasoactive agents, such as phenylephrine to rise and sodium nitroprusside to lower blood pressure. The baroreflex gain is than calculated from the linear portion of these artificially induced blood pressure ramps (Figure 1.). By the **neck chamber method** suction or pressure is applied on carotid sinuses, which stimulates carotid baroreceptors mimicking blood pressure changes. These methods were long considered as the "golden standards". However, it soon became evident that they have several drawbacks regarding their wide clinical use. For the pharmacological method one should insert an intravenous line and give a vasoactive drug, and for the neck chamber method special equipment is necessary. Besides, it is questionable how much these external manipulations influence the very thing, the baroreflex function that we want to investigate.

Recently two new non-invasive methods have been developed to measure baroreflex gain. The first, the **linear spontaneous sequence technique** is based on the occurrence of spontaneous fluctuations in blood pressure accompanied with concordant RR interval changes. First Bertineri analysed spontaneous heart rate and blood pressure fluctuations in animals, and Fritsch applied this technique to humans (Bertineri et al., 1988; Fritch et al., 1986). Today we define spontaneous baroreflex sequences as three or more cardiac cycles with either blood pressure elevation or fall with RR interval changes in the same direction. Search for these sequences allows the calculation of linear regression slope between blood pressure and RR interval changes, the so-called spontaneous BRS, usually separately for up

and down sequences (upBRS, downBRS) (Figure 2.). This technique is easy to use, the only problem is the number of sequences from which we calculate BRS, so the time of the recordings (Waterrich et al., 1998). This problem is especially important in patient groups, where the baroreflex engagement is impaired, so the usual 5 minutes recordings may not contain baroreflex sequences at all.

A complex fluctuation, like the blood pressure and heart rate fluctuations, contains several components. By **spectral analysis** we can assess the frequency distribution of the rhythmic blood pressure and the associated RR interval oscillations (Figure 3.). By Fast Furier Transformation we can separate and characterize the individual waveforms of these signals by their wavelength (frequency) and by their amplitude/power. The cross-spectral method allows studying the interrelation of these two waveforms. The relationship is described by the coherence level, as coherence 1 meaning that the two signals are completely related, coherence 0 meaning no relationship. Alpha index, an index of baroreflex gain can be calculated as the square root of ratios of systolic pressure and RR interval power spectral densities. From the different frequency bands the low frequency (LF) (0,05-0,15 Hz) alpha index is supposed to reflect baroreflex mechanism, and correlates with BRS derived from pharmacological approach (Bernardi et al., 1994; Sleight et al., 1995; Watkins et al., 1996; Airaksinen et al., 1997; Passino et al., 1997; James et al., 1998; Koh et al., 1998; Pitzalis et al., 1998; Rudas et al., 1999). However, several groups use high frequency (HF) (0,15-0,5 Hz) alpha index as a baroreflex parameter which is better regarded as a measure of respiratory sinus arrhythmia.

BRS derived from the **Valsalva manoeuvre** (vBRS) was first described by Pickering and Sleight in 1969 (Pickering and Sleight, 1969). The hemodynamic response to the Valsalva manoeuvre is complex, involving reflexes arising from the lungs, muscles, the low and high pressure baroreceptors. The manoeuvre normally has four phases (Figure 4.). The initial arterial pressure elevation (phase I) is induced by the mechanical transmission of the increased intrathoracic pressure. Impeded venous return in early phase II is reflected in gradually decreasing systolic arterial pressure and pulse pressure. In late phase II arterial baroreflexes mediate compensatory blood pressure elevation. Several studies indicate that the elevation of mean arterial pressure in this phase is the measure of sympathetic baroreflex regulation (Sandroni et al., 1991; Engelke et al., 1995). Phase III is the mechanical result of

sudden intrathoracic pressure drop at the time of releasing the strain. In phase IV the increased venous return and cardiac output and the still constricted arteriolar bed result in blood pressure overshoot and consequently, a vagally mediated bradycardia (Korner et al., 1976; Nishimura and Tajik, 1986; Benarroch et al., 1993; Sandroni et al., 1991; Smith et al., 1996). This phase of the manoeuvre is suitable for cardiac vagal BRS calculation. After Pickering and Sleight the method has been used by several groups with different modifications (Palmero et al., 1981; Smith et al., 1987; Airaksinen et al., 1993; Kautzner et al., 1996; Raczak et al., 2001). Analyses may involve the whole phase IV, or are restricted to segments where the systolic blood pressure exceeds the baseline. According to the analysis of Smith et al. in 1987, the baroreflex gains calculated from the whole phase IV or from the overshoot part of it, can be different. (Smith et al., 1987). The method used by Palmero derived baroreflex gain from the part of phase IV, where good correlation was found between RRI and blood pressure changes (Palmero et al., 1981).

1.3. The problem of normal values and reproducibility

Though heart rate and blood pressure variability (HRV, BPV) parameters and different measures of baroreflex gain are widely used, very few data exist regarding their clinical usefulness. Although the proper function of autonomic reflexes is essential for cardiovascular regulation, very little is known about normal values. Kardos et al. created one of the largest databases till today in a wide age-ranged population (Kardos et al., 2001). In their database they documented a wide scatter of these parameters in healthy subjects during supine rest, even among subjects belonging to the same age group.

It is also well-known that several factors affect these parameters, like posture, the pattern of breathing, volemic state and even emotional stress has major influence on them. Since the so-called "normal" values are dependent on the experimental or clinical setting, we cannot compare data from different study conditions, e.g. data from supine and sitting position, or data during spontaneous and during patterned breathing with a very different respiratory rate. Furthermore, though the new non-invasive techniques have the advantage of examining baroreflex regulation under physiological conditions (no external manipulation occurs), the BRS values derived from different methods are not identical.

1.4. The influence of respiration

In the rhythmic fluctuation of blood pressure and heart rate we traditionally distinguish a slow (around 0,1 Hz) and a rapid (0,2-0,25 Hz) component. This latter is regarded as a consequence of breathing. However, if someone has periodical breathing that will produce another unexpected slow rhythm and will affect spectral analysis (Novak et al., 1994; Mortara et al., 1997). By patterned breathing it is possible to control these individual respiratory alterations and their effects on analysis.

The problem with patterned breathing is whether it itself influences the autonomic regulation. In his study Patwardhan after determining the subjects own respiratory rate, used this frequency for patterned breathing. According to this study controlling of the respiratory rate did not influence the cardiac vagal activity (Patwardhan et al., 1995). However, the situation is not the same, if the frequency of the patterned breathing is different. Slow respiratory rhythm increases LF alpha and spontaneous sequence BRS (Gerritsen et al., 2000; Török et al., 1997; Wichterle et al., 2000). Figure 5. illustrates the effects of different respiratory rates on heart rate and blood pressure variability frequency domain parameters. With 15/min patterned breathing spectral analysis of both waveforms results in a peak around 0,25 Hz. The coherence level is very high in this frequency range, signifying correlation, but not necessarily cause-effect relationship between the two parameters. If we look at the phase angle between the two waveforms, it is around zero at 0,25 Hz, meaning that the fluctuations of heart rate and blood pressure are happening at the same time, as a result of a third parameter, which is respiration (in the case of heart rate it is the well-known respiratory sinus arrhythmia). In contrast, at 0,1 Hz there is also high coherence between the two signals, but the phase angel between them is around -90°. That means that blood pressure changes precede RRI changes, and if we calculate the time delay from the phase angle, it corresponds with baroreflex response time. With 6/min breathing frequency, the high frequency spectral peaks disappeared, but the peaks at 0,1 Hz were exaggerated, because the respiratory and baroreflex mediated spectral components merged (Davies et al., 1999). This mechanism might be one cause of higher BRS values with slow breathing frequency.

1.5. The influence of hypo and hypervolemia

Arterial and cardiopulmonary baroreflexes play an essential role in maintaining blood pressure in acute hypovolemic states. Data from animal studies of volume loading and unloading suggest that cardiopulmonary baroreceptor input can modify arterial baroreflex responses. However, in humans it is not clear what, if any role play the cardiopulmonary baroreflexes in modulating arterial baroreflex control of the circulation. Regarding the baroreflex control of heart rate there are opposing data during central volume changes induced by direct volume manipulation or gravitation induced volume shifts.

It is well-known, that arterial pressure variability increases with hypovolemia. The changes of systolic blood pressure during the respiratory cycle can be described by the systolic pressure variation (SPV) and by its components, the so-called delta up and delta down (Figure 6.). These indices are regarded as sensitive indicators of volemic state (Perel et al., 1987; Rooke, 1995; Rooke et al., 1995). Furthermore, recently by the so-called functional hemodynamic monitoring, we use the mechanical ventilation induced fluctuation in arterial pressure, pulse pressure or stroke volume as a guide for volume responsiveness in the critically ill (Pinsky et al., 2005). Blood pressure fluctuations also can be characterized by frequency domain indices. Using power spectral analysis not just the magnitude, but the frequency distribution of systolic, diastolic and mean blood pressure fluctuations can be assessed. By cross spectral method the feedback relationship of blood pressure and heart rate modulation can also be investigated.

1.6. The aims of the investigations

In our first study we investigated the correlation between the two new non-invasive techniques, the linear spontaneous sequence and the cross spectral methods in measuring baroreflex gain (I.).

In the following study we extended the comparison of cardiac vagal baroreflex gain derived from the two spontaneous methods with the Valsalva manoeuvre derived BRS in a large group of healthy individuals (II.). Baroreflex mediated vasoconstriction was also

assessed by measuring the mean arterial pressure rise in late phase II. In addition, we investigated if these parameters correlated with ageing.

In our next study we assessed the reproducibility and the influence of breathing on the most frequently used heart rate and blood pressure variability and baroreflex indices in two experimental settings, during spontaneous and 6/min patterned breathing (IV.).

To assess autonomic responses to acute volume loss we chose the setting of blood donation (III.). We compared the time and frequency domain parameters of heart rate and blood pressure variability and the baroreflex control of heart rate before and after 350-400 ml blood withdrawal from healthy donors.

2. Materials and Methods

2.1. Subjects

Our study populations consisted of healthy volunteers. 10 young subjects participated in the first study (age 30±7 years, 6 men, 4 women) (I.). The data of 56 healthy subjects were assessed in the study of non-invasive methods and the Valsalva manoeuvre (age 34±11, 18-59 years; 31 men, 25 women) (II.). In the reproducibility study we investigated 10 healthy volunteers (25±2 years, 3 women, 7 men) (IV.). None of these subjects had any medical problems and none of them took any medication. In addition, we asked them to refrain from cigarette smoking and consuming caffeine containing beverages before the studies. In the blood donation trial the study population consisted of 48 healthy volunteers (age 35±12, 18-59 years; 23 men, 25 women), who were recruited when they signed up for blood donation (III.). Three of them were first time, the others were regular blood donors. None of the subjects had a previous history of syncope or presyncope. All the subjects were informed and gave their consent for their participation in the investigations.

2.2. Measurements

2.2.1. Study conditions and equipment

All the measurements were made in a quiet room after 5-10 minutes supine rest. In all studies the ECG and blood pressure signals were continuously measured with a Marquette bedside monitor and with the **Finapres** 2300 non-invasive blood pressure monitor. The breathing was recorded by the means of a pneumatic rubber belt (pneumobelt) which was applicated around the subjects' abdomen. This device was connected to a pressure transducer and to an analog-digital converter. The central frequency of this uncalibrated breathing signal was assessed by the spectral method. All signals were recorded on-line and digitalized with 500 Hz by the **Dataq/Windaq** system. We always took 5-5 minutes recordings of ECG, blood pressure and uncalibrated breathing signals. Data were analysed off-line using our self developed software in the first study (Gingl Z), and the commercially available **WinCPRS** (Absolute Aliens Ay, 2000) software in the others. All recordings were peak detected automatically and checked by one of the investigators (this means that all R waves on the ECG signals and all systolic peaks and diastolic valleys on blood pressure signals were marked, to enable further calculations). We discarded recordings which contained premature beats or artefacts, because these seriously disturb analysis, especially the spectral method.

2.2.2. The linear spontaneous sequence method

After the confirmation of peak detection search for spontaneous sequences and power spectral analysis of RR intervals and systolic arterial pressure (SAP) were conducted. Spontaneous sequences were defined as three or more consecutive cycles of either SAP elevation (the so-called up sequences) or fall (down sequences) coupled with RRI changes in the same direction. The sensitivity of blood pressure change was set to 1 mmHg/heartbeat. No limit to RR interval changes was applied in order not to bias the results toward higher BRS values. When the sequences were selected, linear regression analysis was performed, and only sequences with correlation coefficient >0,8 were accepted for further analysis. A phase shift of one cycle (RR interval of the next cycle which immediately followed systolic blood pressure change) was used to generate data points in our first study, later the phase shift was automatically set by the computer program according to the Yamamoto-Hughson method (Hughson et al., 1993). For all of these sequences the slope of the line fitted to delta RRI versus delta SAP was calculated and averaged separately for up and down sequences. This

calculation was performed only if 5-5 or more sequences were identified during the 5 minutes recordings.

2.2.3. The cross spectral analysis

Power spectral analysis was made for RRI and SAP fluctuations by using fast Fourier transformation. To assess the association between RRI and SAP powers cross spectral analysis was performed, and only data with coherence >0,5 were used for alpha index calculation. Cross spectral alpha index was calculated as the square root of ratios of SAP and RRI powers. It was derived only in the low frequency band (0,05-0,15 Hz) in the first two studies, because only low frequency alpha index (LF alpha) is regarded as an estimate of baroreflex gain. When we assessed the effects of respiratory frequency and volume unloading, we also calculated high frequency (0,15-0,5 Hz) alpha index (HF alpha), because here we expected changes due to the interventions. In the blood donation study we also assessed if the phase angle (which correlates with the time relationship of the two signals) between the SAP and RRI signals changed during the two study conditions (LF angle, HF angle).

2.2.4. The Valsalva manoeuvre

Valsalva manoeuvres were performed twice at a pressure of 30-40 mmHg for 15 sec, in supine position. The subjects blew into a plastic tube which was connected to an aneroid manometer. A small leak in the system prevented the maintenance of the expiratory pressure by occluding the glottis. The vBRS was derived from the phase IV of the best manoeuvre using a technique similar to that described by Palmero et al (Palmero et al., 1981). According to this, we used a given part of the whole phase IV (from the smallest blood pressure after releasing the strain to the highest value during the overshoot phase), where the criteria used for spontaneous sequence selection was fulfilled. In addition, the mean arterial pressure (MAP) elevation was calculated from the late phase II of the manoeuvre.

2.2.5. Time and frequency domain indices of heart rate and blood pressure variability

In the reproducibility and blood donation study we calculated the classic heart rate and blood pressure variability indices. The time domain indices were the following: mean RR interval; SDRR (the standard deviation of RR intervals); the RMSSD (the root mean square of successive differences of RRI) and the PNN50 (the percentage of cardiac cycles, where the difference was greater than 50 msec between the successive beats); for blood pressure variability the mean systolic, diastolic and mean arterial pressure (SAP, DAP, MAP); and the standard deviation of these. Besides, frequency domain parameters were also assessed as the low and high frequency power spectral densities of RRI and SAP (RRI LF, RRI HF, SAP LF, SAP HF) (Task Force of the ESC and NASPE).

2.3. Study protocols

In the first study 5-5 minutes periods of controlled breathing with 15/min breathing frequency were recorded and from these the spontaneous sequence and cross spectral BRS values were derived and compared (I.).

In the second study first, 5 minutes resting recordings were made during patterned breathing at a rate 15/min (0,25Hz). After that the subjects were asked to perform two Valsalva manoeuvres. From these data the spontaneous sequence, the cross spectral and the Valsalva BRS, and the elevation of MAP in late phase II. of the Valsalva manoeuvre were calculated II.).

In the reproducibility study the measurements were made and were repeated on 10 consecutive days (IV.). For each subject on each day 5-5 minutes ECG and blood pressure signals were recorded during spontaneous and 6/min patterned breathing. The order of these breathing patterns was always the same. In this study we compared the heart rate and blood pressure variability parameters, and the spontaneous sequence and cross spectral BRS values and their repeatability during spontaneous and 6/min patterned breathing. Besides, we examined if the controlling of breathing had any influence on the number of spontaneous sequences (BRS validity) (Penttilä et al., 2001). We also assessed if the phase angle between the SAP and RRI signals changed during the two study conditions (LF angle, HF angle).

In the blood donation study first, 5-5 minutes baseline recordings were taken (III.). This was followed by the venipuncture and by the withdrawal of 350-400 ml blood in

approximately 5 minutes. After the removal of the needle, we started at once with the second recordings. The subjects were not allowed to drink, nor received caffeinated beverages throughout the investigations. Two of them experienced mild symptoms suggesting vasovagal reaction, they were put to head-down tilt position at the end of the blood withdrawal, according to the protocol of the Blood Bank. By the time of the second recording, when their condition stabilized, they were put back supine, therefore their data were not excluded from analysis. From the recordings taken before and after the blood withdrawal the classic time and frequency domain parameters of HRV and BPV, and the two non-invasive BRS indices were calculated, and compared between the two study conditions.

2.4 Statistical analysis.

For all studies data are given as mean±SD if not otherwise indicated. In the first study for comparison of BRS estimates derived from up sequences, down sequences and low frequency alpha index Friedman repeated measures analysis of variance was used (I). To assess the relationship between these parameters linear regression analyses were performed. Differences were considered statistically significant, if p value was <0,05.

In the second study to assess the relationship between upBRS, downBRS, LF alpha and vBRS; and between these parameters and age, linear regression analyses were performed (II). Linear regression analysis was also used in the investigation of the relationship between vBRS and late phase II MAP elevation. P value was set at <0,05 to be considered statistically significant.

In the reproducibility study we compared all the heart rate and blood pressure variability measures and the different baroreflex indices during the two study conditions by using paired t-test (IV.). If the data were not normally distributed, Wilcoxon Signed Rank Test was used. To assess reproducibility within and between-subject variability was calculated as suggested by Lawrence (Lawrence et al., 1992). Standard deviation (SD) of a given parameter was determined from all 5 minutes recordings of the same subject on 10 different days. The average of these values for the whole study group was defined as within-subject SD. SD of a given parameter derived from the recordings of all subjects on the 1st, 2nd etc. days was also determined. The average of the 10 days was defined as between-subject

SD. Within and between-subject SDs were separately calculated for both spontaneous and for 6/min patterned breathing. The ratio of SD within and SD between was used to characterize the reproducibility. The smaller this value, the better the parameters discriminative value is. In addition, because some of the investigated parameters had not normal distribution (that questions the use of SD to describe them) we calculated intraclass correlation coefficient (ICC). We used one-way random effect model repeated measures analysis of variance. Both the single measures (ICCs) and the average measures ICC (ICCa) is given separately for the two study conditions. Reproducibility was considered good, if ICCa was >0,8; moderate, if ICCa was between 0,6 and 0,8; and weak, if it was below 0,6.

In the blood donation study the measured parameters before and after blood withdrawal were compared by paired t-test, and when the data were not normally distributed, Wilcoxon signed rank test was used (III.). P value was set at <0,05 to be considered statistically significant.

3. Results

3.1. Comparison of different non-invasive baroreflex indices

3.1.1. Spontaneous sequence and cross-spectral methods (I.)

In this study the spontaneous sequences (up+down) represented 41,2% (range 9,7-93,6%) of total cardiac cycles. LF alpha index was impossible to calculate in one subject due to the low level of coherence between RRI and SAP powers. The BRS values calculated from up sequences, down sequences and low frequency alpha index were 13,0±8,6 msec/mmHg, 10,3±5 msec/mmHg and 9,5±5 msec/mmHg, respectively. LF alpha index was lower than BRS derived from up and down sequences, however these differences did not reach statistical significance. Up and down BRS values were closely related (R=0,92, p<0,001). LF alpha index showed moderate correlation only with upBRS (R=0,7, p=0,04).

3.1.2. Spontaneous sequence, cross-spectral and the Valsalva manoeuvre derived baroreflex gain (II.)

The BRS values calculated by different methods are summarised in Table 1. The upBRS was impossible to calculate in 6, the downBRS in 12, LF alpha in 4 and vBRS in 7 individuals.

upBRS (msec/mmHg)	12±8,6
downBRS (msec/mmHg	10±6,1
LF alpha (msec/mmHg)	12,1±8,2
vBRS (msec/mmHg)	9,7±7,2

Table 1. Baroreflex gain values calculated from up sequences (upBRS), down sequences (downBRS), from phase IV of the Valsalva manoeuvre (vBRS) and low frequency alpha index (LF alpha) (mean±SD).

We found close correlation between the baroreflex gain derived from up and down sequences (R=0,91, p<0,001); and down sequences and low frequency alpha index (R=0.81, p<0,001). The correlation was also significant between upBRS and LF alpha (R=0,65, p<0,001). There was a weak, significant correlation between vBRS and downBRS (R=0,37, p=0,043), but no correlation between vBRS and upBRS; and vBRS and LF alpha.

All the baroreflex gains derived from different methods showed significant negative correlation with age (upBRS: R=0,42, p=0,005; downBRS: R=0,36, p=0,031; LF alpha: R=0,39, p=0,009; vBRS: R=0,41, p=0,003).

The MAP elevation in late phase II was 17±11 mmHg (range: 0-52 mmHg) and was not influenced by increasing age. Similarly, the vBRS showed no correlation with the MAP increase in late phase II.

3.2. The reproducibility of the parameters of human cardiovascular autonomic regulation (IV.)

For the final analysis we used data from 8 subjects, because the quality of the recordings of 2 excluded proper peak detection. Table 2. and 3. contain the within and between-subject SD-s of the parameters, and the within/between SD ratio for spontaneous and

6/min patterned breathing. The between-subject SD was always greater than the within subject SD (except for SAP LF during 6/min breathing), and this ratio decreased during 6/min controlled breathing for SDRR, RMSSD, RRI LF, upBRS and downBRS.

	within SD	between SD	within/between ratio
RRI (msec)	64,9	127,7	0,51
SDRR (msec)	10,8	22,4	0,48
RMSSD (msec)	14,1	30,3	0,47
PNN50 (%)	9,4	22,5	0,42
RRI LF ($msec^2$)	429	643	0,67
RRI HF ($msec^2$)	701	1928	0,36
SAP LF ($mmHg^2$)	3,9	4,5	0,87
SAP HF ($mmHg^2$)	1,3	2,0	0,65
UpBRS (msec/mmHg)	4,31	7,07	0,61
DownBRS (msec/mmHg)	4,46	7,49	0,60
LF alpha (msec/mmHg)	4,11	6,27	0,66
HF alpha (msec/mmHg)	6,7	13,3	0,50

Table 2. Within and between-subject variability and the within/between ratio for the assessed parameters during spontaneous breathing.

	within SD	between SD	within/between ratio
RRI (msec)	58,3	112,5	0,52
SDRR (msec)	13,3	33,5	0,40
RMSSD (msec)	12,4	30,4	0,41
PNN50 (%)	9,1	17,8	0,51
RRI LF ($msec^2$)	1898	4604	0,41
RRI HF ($msec^2$)	274	716	0,38
SAP LF ($mmHg^2$)	7,7	7,6	1,01
SAP HF ($mmHg^2$)	0,6	0,7	0,86
UpBRS (msec/mmHg)	6,1	13,3	0,46

DownBRS (msec/mmHg)	3,48	6,45	0,54
LF alpha (msec/mmHg)	4,94	7,49	0,66
HF alpha (msec/mmHg)	8,7	15,0	0,58

Table 3. Within and between-subject variability and the within/between ratio for the assessed parameters during 6/min patterned breathing.

ICCa showed good or moderate reproducibility for all parameters, except blood pressure (SAP, DAP, MAP, SAP SD), that did not improve further with controlling the breathing at the rate of 6/min (Table 4.). However, because of missing values, in the case of upBRS, downBRS and LF alpha during spontaneous breathing ICCa was calculated on so few cases, that seriously questions its validity.

	spontaneous breathing		6/min breathing	
	ICCs	ICCa	ICCs	ICCa
RRI	0.712	0.961	0.710	0.961
SDRR	0.745	0.967	0.821	0.979
RMSSD	0.753	0.968	0.810	0.977
PNN50	0.782	0.973	0.696	0.958
SAP	0.062	0.400	0.115	0.565
SAP SD	0.254	0.773	0.108	0.548
DAP	0.094	0.508	0.287	0.801
MAP	0.052	0.356	0.174	0.678
RRI LF	0.407	0.873	0.787	0.974
RRI HF	0.729	0.964	0.765	0.970
SAP LF	0.203	0.718	-0.061	-1.367
SAP HF	0.367	0.853	0.234	0.753
UpBRS	0.757	0.969	0.774	0.972
DownBRS	0.820	0.978	0.645	0.948
LF alpha	0.352	0.844	0.588	0.935
HF alpha	0.700	0.959	0.683	0.956

Table 4. Intraclass correlation coefficients (ICCs: single measure; ICCa: average measures) for the investigated parameters during spontaneous and 6/min patterned breathing.

3.3. The influence of respiration on autonomic cardiovascular indices (IV.)

Table 5. contains mean values and within-subject SD of the 10 measurements for each subject during spontaneous and 6/min patterned breathing. Comparing values in the two study conditions we found significant change in almost all parameters, except the mean heart rate and SAP, and the phase angles between RRI and SAP both in LF and HF. The variability of RRI and SAP increased with 6/min breathing as it was seen in the changes of SDRR, PMSSD, PNN50 and SAP SD. This increased variability was accompanied by increased fluctuation in LF band. At the same time the HF power of RRI and SAP decreased.

UpBRS, LF alpha and HF alpha increased significantly with 6/min patterned breathing, but down BRS did not change. The number of spontaneous sequences also increased during patterned breathing, as the increased percent of up and down sequences showed it. The phase angle between SAP and RRI did not change between the settings.

	spontaneous breathing	6/min breathing	p values
RRI (msec)	911±124	906±109	NS
SDRR (msec)	48±22	77±33	<0,001
RMSSD (msec)	43±29	51±30	<0,001
PNN50 (%)	18±21	24±17	=0,001
SAP (mmHg)	131±18	129±20	NS
SAP SD (mmHg)	6,2±1,9	7,0±1,9	<0,001
DAP (mmHg)	73±14	70±14	<0,001
MAP (mmHg)	92±16	88±16	<0,001
RRI LF ($msec^2$)	694±702	5204±4540	<0,001
RRI HF ($msec^2$)	1101±2027	543±728	<0,001
SAP LF ($mmHg^2$)	7,2±4,6	18,6±8,1	<0,001
SAP HF ($mmHg^2$)	2,1±2,6	0,95±0,95	<0,001

LF angle	-45,4±28,6	-48,2±22	NS
HF angle	-4,6±23,5	-7,3±54	NS
UpBRS (msec/mmHg)	12,2±8,3	16,3±12	<0,001
DownBRS (msec/mmHg)	12,8±8,2	13,3±6,3	NS
Up sequences (%)	12,4±8,8	32±6,8	<0,001
Down sequences (%)	14,1±16,3	35,2±7,9	<0,001
LF alpha (msec/mmHg)	12,4±6,8	17,8±7,6	<0,001
HF alpha (msec/mmHg)	20,4±13,2	23,9±15,2	=0,012

Table 5. Mean±SD for the investigated HRV, BPV parameters and baroreflex indices during spontaneous and 6/min patterned breathing.

3.4. The effects of volume loss on autonomic cardiovascular indices (III.)

The systolic, diastolic and mean arterial blood pressure increased after the blood withdrawal. The mean heart rate and the central frequency of the uncalibrated breathing signal did not change (the latter was 0,26 Hz). From the time domain indices of RRI RMSSD significantly decreased, SDRR and PNN50 did not show any change (Table 6.).

	before	after	p value
SAP (mmHg)	124±16	128±13	<0,001
DAP (mmHg)	74±13	76±12	<0,001
MAP (mmHg)	91±13	93±12	p=0,003
RRI (msec)	816±142	806±138	NS
SDRR (msec)	41±24	40±22	NS
RMSSD (msec)	33±25	28±26	p=0,027
PNN50 (%)	3,7±0,1	1,0±0,2	NS

Table 6. The systolic (SAP), diastolic (DAP) and mean (MAP) arterial pressure, the mean RR interval (RRI), standard deviation of RRI (SDRR), root mean square of successive differences (RMSSD) and PNN50 before and after blood donation.

SAP variability increased in both frequency bands, but it reached statistical significance only in the high frequency band after blood withdrawal. The DAP variability increased significantly in both frequency ranges. Heart rate variability in average increased in LF and tended to decrease in HF band, however the individual responses showed substantial diversion in this range (Table 7.).

	before	after	p value
RRI LF ($msec^2$)	221*	330*	p=0,025
SAP LF ($mmHg^2$)	4,8±3,9	5,8±3,9	p=0,071, NS
DAP LF ($mmHg^2$)	2,1±1,5	2,7±1,8	p=0,017
RRI HF ($msec^2$)	297*	172*	p=0,063, NS
SAP HF ($mmHg^2$)	1,2*	1,9*	p<0,001
DAP HF ($mmHg^2$)	0,7±0,8	1,0±0,8	p=0,013

Table 7. Spectral powers in low frequency (LF) and high frequency (HF) bands of RR intervals (RRI), systolic (SAP) and diastolic (DAP) blood pressures before and after blood donation. (*indicates median values for not normally distributed data)

From the different baroreflex gain estimates upBRS and HF alpha index decreased significantly due to the intervention, the decrease in downBRS did not reach statistical significance, and the LF alpha showed no consistent change at all (Table 8.).

	before	after	p value
upBRS (msec/mmHg)	12,0±8,6	9,6±7,2	p=0,001
downBRS (msec/mmHg)	10,0±6,1	8,0±6,1	p=0,098, NS
LF alpha (msec/mmHg)	9,2*	9,2*	NS
HF alpha (msec/mmHg)	19,4±14,8	13,2±11,1	p=0,001

Table 8. Cardiac vagal baroreflex gain values derived from up sequences (upBRS), down sequences (downBRS), low frequency alpha index (LF alpha) and high frequency alpha index (HF alpha) before and after blood donation. (*indicates median values for not normally distributed data)

The phase angle between RRI and SAP powers in LF did not change due to the volume loss (-58±24° and -54±26°). In the HF range the phase became more negative after blood donation (-1±29° and -17±32°, p=0,001).

4. Discussion

4.1. Comparison of different non-invasive baroreflex indices (I., II.)

In our first two studies we evaluated two non-invasive methods in BRS estimation based on 5 minute resting recordings for analysis. These techniques examine spontaneous fluctuations and rhythmicity of RRI and blood pressure, so making it possible to calculate baroreflex gain in the narrow range around the resting operation point, which is located in healthy volunteers on the linear portion of the RRI blood pressure relationship (Figure 7.). In the pharmacological method by using either phenylephrine or nitroprusside the blood pressure changes are greater than during spontaneous fluctuations, and these changes may occur along the saturation or the threshold range of the sigmoid relationship, so the calculated baroreflex indices might be different.

Studies comparing BRS derived from spontaneous sequence and pharmacological methods found good correlation, although the absolute values were different (Hughson et al., 1993; Parlow et al., 1995; Watkins et al., 1995; Watkins et al., 1996; James et al., 1998; Pitzalis et al., 1998; Rudas et al., 1999).

In our first study BRS estimated from down sequences tended to be lower than BRS derived from up sequences, similarly to the relationship found between sodium nitroprusside and phenylephrine BRS (Parlow et al., 1995; Rudas et al., 1999) (I.). It seems, that raising blood pressure is followed by a larger baroreflex response than falling blood pressure.

However, the up and downBRS were closely related to each other in our study similarly to others (James et al., 1998; Pitzalis et al., 1998; Rudas et al., 1999).

By **cross spectral analysis** we only examined data in the low frequency range. Several studies suggested that oscillations in this band are related to baroreflex mechanism, and these oscillations are caused by the phase lag (delay) in the baroreflex loop (Bernardi et al., 1994; Sleight et al., 1995; Koh et al., 1998; Passino et al., 1997). According to this hypothesis alterations in blood pressure cause a fast vagal (<1 sec) response to the heart and a slow sympathetic response (approximately 10 sec) to the vessels. This in turn alters heart rate producing a rhythm with frequency 0,1Hz. This peak in RRI LF power can be decreased or eliminated by Atropine, sino-aortic denervation or epidural anaesthesia (Di Rienzo et al., 1997; Jokkel et al., 1995; Parlow et al., 1995; Scheffer et al., 1994). Others emphasise, that these low frequency oscillations also represent central rhythmicity because they are present during stable, unperturbed conditions (Koh et al., 1998; Passino et al., 1997) (remarkably with a little bit lower frequency than 0,1Hz). In contrast, high frequency (respiratory) fluctuations do not seem to involve baroreflex mechanism. In part they are due to the mechanical effects of respiration on blood pressure, and vagally mediated to the heart (Koh et al., 1998; Parati et al., 1995). In order to separate LF and respiratory bands we controlled the breathing of our subjects at a rate of 15/min (0,25Hz).

Studies comparing **spectral alpha index with pharmacological BRS** show large variations in methods. Some of them use LF, some both low and high frequency alpha index, and certain groups average LF and HF to one spectral alpha index, making data interpretation difficult. In spite of this the spectral BRS shows good correlation with pharmacological BRS, also there are differences in absolute values (Airaksinen et al., 1993; Watkins et al., 1996; Passino et al., 1997; James et al., 1998; Rudas et al., 1999).

There are only a few published studies regarding the **relationship between linear sequence method and spectral BRS.** Good correlation was found in several studies (James et al., 1998; Watkins et al., 1996; Pitzalis et al., 1998; Rudas et al., 1999), with a good agreement in the study of James. In our first study LF alpha index showed only moderate correlation with upBRS (I.). This finding may be partly due to the small number of data. In our second study LF alpha index showed good correlation both with up and downBRS (II.).

Since it was first proposed, several groups used the phase IV of the **Valsalva manoeuvre** for cardiac vagal baroreflex determination. The methods used were different, some calculating BRS from the whole phase IV, some just from the overshoot phase of it, where systolic blood pressure exceeded the baseline value (Palmero et al., 1981; Smith et al., 1987; Airaksinen et al., 1993; Raczak et al., 2001). The phase lag used between RRI and blood pressure signals was also different. However, in all studies vBRS correlated significantly with the phenylephrine method (Palmero et al., 1981; Smith et al., 1987; Airaksinen et al., 1993; Raczak et al., 2001). The agreement between values changed according to which part of phase IV was used for calculation. In the study of Smith et al., the BRS from the whole phase IV significantly underestimated, while that from the overshoot phase significantly overestimated baroreflex gain compared to phenylephrine method (Smith et al., 1987). In a recent study Raczak et al. published similar findings, and better agreement with phenylephrine method, if the overshoot phase was used (Raczak et al., 2001).

We chose the whole phase IV for baroreflex gain calculation because it seems that BRS values are comparable if blood pressure elevation starts from the resting value or from below it (Rudas et al., 1999). The problem of phase lag was solved by the software used because it selected sequences with the best correlation coefficient (II).

The phase IV of the Valsalva manoeuvre involves other mechanisms in heart rate changes besides vagal slowing. The sympathetic cardioacceleration present in late phase II also has an influence on blood pressure elevation in phase IV, and may alter the heart rate response. The intravascular and intrathoracic blood volumes, the end expiratory pressure of straining and body position are also important determinants of hemodynamic responses to the Valsalva manoeuvre.

From the few studies dealing with the **comparison of spontaneous methods and the Valsalva BRS** determination, our group was among the first one to describe the relationship between linear sequence and the Valsalva-derived baroreflex gains (Kardos et al., 1997). Lord et al. published data about the correlation of cross spectral alpha index and the Valsalva BRS (Lord et al., 1998). The above mentioned physiological factors may partly explain the weak correlation found in our current study between vBRS and downBRS, and that no correlation was found between vBRS and upBRS and LF alpha (II.)

There are several reports indicating that arterial **baroreflex regulation of efferent sympathetic and vagal activities** are neither strictly complementary, nor closely parallel processes. Rudas et al. found no relationship between vagal and sympathetic baroreflex gains (Rudas et al., 1999). Blood pressure regulation in the late phase II of the Valsalva manoeuvre is a complex process. Beat-to-beat decreases in stroke volume are counterbalanced by increasing sympathetic activity (as reflected by muscle sympathetic nerve activity), and in turn increased vascular resistance. The net result of these processes, the late phase II MAP elevation, could be regarded as a surrogate of sympathetic responses (Sandroni et al., 1991). In the present study, in line with previous findings, we found no correlation between the Valsalva derived cardiac vagal BRS and the MAP change in late phase II (II.).

Bristow first published in 1969 that cardiac vagal baroreflex gain decreases with **ageing** (Bristow et al., 1969). From this time several groups reached the same conclusion, using phenylephrine (Laitinen et al., 1998; Ingall et al., 1990), the Valsalva manoeuvre or the spontaneous methods (Shimada et al., 1986; Airaksinen et al., 1993; Siche et al., 1995; Baevski et al., 2000). The study of Rudas et al. widened this observation for the BRS calculated from the nitroprusside method (Rudas et al., 1999). Recently, the study of Kardos et al. also proved the negative correlation between cardiac vagal baroreflex gain and age, and provided a large database for spontaneous sequence baroreflex gain for healthy individuals (Kardos et al., 2001).

In contrast, sympathetic baroreflex gain seems to be independent of ageing (Ebert et al., 1992; Matsukawa et al., 1996; Rudas et al., 1999). In accordance with it is our finding, that the sympathetically mediated mean arterial pressure elevation in late phase II of the Valsalva manoeuvre did not show age-related changes (II.).

There are certain features which **limit the clinical utilisation** of the spontaneous methods. In certain individuals the small number of sequences prevents calculation of up or downBRS and the low level of coherence prevents calculation of alpha index. Also, cross spectral analysis cannot differentiate between responses to rising and falling blood pressure. Furthermore, spectral analysis is extremely artefact sensitive. The Valsalva manoeuvre requires the co-operation of the patient, and in certain clinical circumstances performing the manoeuvre is contraindicated. The so-called "square wave" response or the lack of significant

blood pressure changes in certain diseases also makes the widespread use of the manoeuvre difficult.

4.2. The reproducibility of the parameters of human cardiovascular autonomic regulation (IV.)

The main finding of our study is that most of the commonly used heart rate and blood pressure variability parameters and BRS indices show large intra- and inter-individual variability, even during controlled laboratory conditions, however most of them have acceptable reproducibility during spontaneous and 6/min patterned breathing. Though respiration has great influence on these parameters, and slow patterned breathing improves the validity of spontaneous sequence BRS, it does not seem to consistently improve their reproducibility.

Does it seriously question the usefulness of these parameters in describing autonomic dysfunction or in risk stratification? I think, no. We do need more data on the behaviour of these parameters during different conditions, so it would be possible to define normal ranges for physiological changes. It is well-known, that **HRV and BPV parameters** are largely influenced by several conditions, the most important of these are posture and respiration (Cooke et al., 1999; Koh et al., 1995). In spite of this, not all the studies dealing with the topic control or monitor these circumstances. The problem is especially difficult to solve in the case of long-term (ambulatory) recordings. During daily activities the rate and tidal volume of respiration, the degree of physical activity, the emotional or stress state, the volemic state of the subjects change continuously. So it is really not easy to tell what we can predict from these measurements. Regarding the short-term recordings, it is absolutely necessary and usually possible to control or at least describe precisely the study conditions before drawing conclusion about clinically important changes. It is especially important in the case of calculating frequency domain parameters.

The value of **different baroreflex indices** is also problematic. We know that the different methods for calculating BRS give different absolute values with different degrees of correlation (Hughson et al., 1993; Parlow et al., 1995;, Watkins et al., 1995; Watkins et al., 1996; James et al., 1998; Pitzalis et al., 1998; Rudas et al., 1999). It is also evident, that

baroreflex function changes continuously during varying activities. We have to adjust the cut-off values of clinically significant impairment according to the type of test and laboratory circumstances.

Another important source of difference comes from the number of individual BRS values used for calculation. In the case of the phenylephrine or the nitroprusside method, we calculate BRS from one ramp of blood pressure elevation or fall. When calculating BRS from the pharmacological methods, investigators use different number of individual measurements for averaging. Drug-induced ramps track a relatively wide range of arterial pressure changes. The same is the case with the neck-chamber method. In contrast, the spontaneous sequence method tracks only a very narrow blood pressure range. Unlike the long ramps induced by drugs or the neck chamber, the short spontaneous sequences may represent chance relationship. The smaller the number of sequences used for averaging, the higher is the confounding effect of a false sequence. Therefore, reliability of the sequence method is related to the sequence number. However, it is not well-defined how many sequences are acceptable to use for BRS calculation.

Making very difficult to interpret the few data available regarding the **reproducibility of HRV and BPV parameters and BRS indices**, is the fact, that different authors use different statistical terms to describe it, e.g. standard deviation, range of standard deviation, coefficient of variation, intraclass coefficient of variation, within- and between subject variability and even Bland-Altman plot (Lawrence et al., 1992; Iellamo et al., 1996; Herpin and Ragot, 1997; Lord et al., 1998; Davies et al., 1999).

We chose the method used by Lawrence, who studied the reproducibility of four commonly used autonomic tests (deep breathing, Valsalva manoeuvre, standing up and normal relaxed breathing in supine position) and repeated them twice on four different occasions over a four week period (Lawrence et al., 1992). They calculated the within and between-subject SD for each test and each day, then they took the ratio of within and between-subject SDs. They concluded that different subjects and different tests had various repeatability, and the main source of poor repeatability was variation in respiration.

Therefore we examined whether controlling the breathing frequency improved reproducibility. Though 6/min patterned breathing resulted in significant change in almost all parameters, it did not consistently affect reproducibility. The repeatability of SDRR, RMSSD,

RRI LF improved (the ratio of within and between-subject SDs decreased), but in the case of pNN50, RRI HF, SAP LF, SAP HF the direction of the change was the opposite. Looking at the BRS indices, the reproducibility of upBRS and downBRS improved, but that of LF alpha did not change at all. When we assessed reproducibility by ICC during spontaneous breathing, we found good reproducibility (ICCa>0.8) of RRI, SDRR, RMSSD, PNN50, RRI LF, RRI HF, SAP HF, upBRS, downBRS, LF alpha, HF alpha; moderate reproducibility (ICCa between 0.6-0.8) of SAP SD, SAP LF. Because of missing values, the number of subjects was very low in the case of upBRS, downBRS and LF alpha, so we could not interpret results regarding these BRS parameters during spontaneous breathing. During 6/min controlled breathing the reproducibility of the time and frequency domain parameters of HRV and BPV and HF alpha did not improve further. In contrast, we found good reproducibility for upBRS, downBRS and LF alpha, mainly as a result of no missing values.

Iellamo studied 20 healthy volunteers and calculated spontaneous BRS at rest, during active standing, during performing mental arithmetic's and during static hand gripping (Iellamo et al., 1996). They repeated the tests on two separate days. They found that during active standing the number of spontaneous sequences increased, but the coefficient of variation did not differ significantly between the test conditions and between the two days. Overall, the reproducibility was high in all four test conditions. They also raised the attention to the fact that the gain of baroreceptor heart rate relationship showed continuous variation even during brief time periods.

Herpin and Ragot investigated the mid and long-term reproducibility of the non-invasive measures of BRS, the spontaneous sequence and the cross-spectral method (Herpin and Ragot, 1997). In 14 healthy volunteers they repeated the measurements at 1 week and at 1 year, both in supine and standing positions. They calculated repeatability coefficients and intraclass correlation coefficient. For spontaneous sequences they found satisfactory reproducibility both for mid and long term, for cross-spectral method it was better at mid than at long-term. Standing position increased the reproducibility for both methods.

Pitzalis investigated the reproducibility of time and frequency domain parameters of heart rate variability in 18 healthy volunteers by repeating the measurements at 2 weeks and at 7 month (Pitzalis et al., 1996). They calculated intraclass correlation coefficient, and concluded that all time domain parameters of HRV showed good reproducibility, except

SDRR, but reproducibility of the frequency domain parameters depended on study conditions; LF power was reproducible under resting, controlled breathing (16/min) and head up tilt, total power only at rest, and HF power only during 16/min controlled respiration.

Davies assessed short-term reproducibility of BRS calculated from the phenylephrine method, cross spectral LF alpha, HF alpha, spontaneous sequence method and the influence of 0.1 Hz controlled breathing in 31 congestive heart failure patients and 18 normal controls (Davies et al., 1999). They used the coefficient of variation to describe reproducibility and found that controlling the breathing resulted in the best reproducibility. In a similar setting we could not document the same trend for LF alpha and HF alpha.

Lord also investigated the reproducibility of three different methods in measuring BRS in healthy subjects, the phenylephrine method, the Valsalva manoeuvre and the cross-spectral method (Lord et al., 1998). They repeated the measurements on three occasions at least one week apart. The coefficient of variation was the best for LF alpha and the worst for vBRS.

The above studies made attempts to standardize different study conditions. Since it is quite true, that human beings spend substantial amount of their time in upright position, it seems reasonable to perform autonomic studies in this posture. Unfortunately, orthostasis is not feasible for several patient populations including those where abnormal BRS proved to be informative. The other factor assessed in the above studies is respiration. We believe, that controlled breathing is essential for HRV and BPV and BRS studies. It is quite clear, that spontaneous respiration can assume various abnormal patterns, like Cheynes-Stockes respiration. This can profoundly alter especially the frequency domain parameters. In addition, as Koh et al. reported, the rate of spontaneous breathing can change substantially between measurements even in healthy young subjects (Koh et al., 1995). Although, we could not demonstrate improved reproducibility of all of these parameters in healthy young subjects, this might be more evident in the case of pathological conditions.

With slow patterned breathing baroreflex mechanisms and respiratory sinus arrhythmia merge, resulting in augmented BRS values. By this method we do not measure "pure" arterial baroreceptor reflex (Davies et al., 1999), but at least we avoid the unpredictable alterations caused by anomalous breathing patterns.

4.3. The influence of respiration on autonomic cardiovascular indices (IV.)

According to our study there were significant changes in almost all parameters of HRV and BPV when we compared spontaneous with slow patterned breathing. With low respiratory rate, especially with 6/min frequency (0,1 Hz) baroreflex mediated fluctuations merge with respiration-induced rhythms. In some patient populations this exaggerated response might be necessary for the evaluation of cardiac vagal baroreflex function (e.g. in Parkinson disease, in heart failure) (Bernardi et al., 2002; Halámek et al., 2003; Oka et al., 2003). Furthermore, with slow patterned breathing the number of spontaneous sequences also increases, thus improving the validity of the linear spontaneous sequence method (Figure 8.).

In conclusion, slow patterned breathing improves the clinical utility of the spontaneous methods and also might help to standardize the experimental conditions under which we investigate BRS indices.

4.4. The effects of volume loss on autonomic cardiovascular indices (III.

The main findings of this study were the following. First, the systolic, diastolic and mean blood pressure increased and the blood pressure variability, as it was expected, increased during volume loss. The increase in systolic blood pressure variability mainly occurred in HF band, where the RRI variability tended to decrease. The increase in SAP variability did not reach statistical significance in LF range, where the RRI variability increased. Second, from the different cardiac vagal baroreflex gain indices the upBRS and HF alpha index decreased significantly. In the LF band RRI-SAP phase angle remained fairly constant before and after blood donation at around -50-60°. This value is compatible with baroreflex mechanism. Phase angles in the HF range significantly decreased from -2 to -17° after the intervention. These values do not support baroreflex transduction.

4.4.1. Mean values, heart rate and blood pressure variability

The mean heart rate did not change due to blood withdrawal. The increase in systolic, diastolic and mean arterial pressures supports the possibility of sympathetic vasoconstriction

due to rapid volume loss, so the decreased stroke volume and cardiac output did not result in hypotension. This finding also suggests that the induced volume changes were great enough to stimulate or alter autonomic responses.

It is known, that arterial pressure fluctuations increase during hypovolemia, especially during mechanical ventilation (Rooke, 1995; Rooke et al 1995; Perel et al., 1997; Ornstein et al., 1998). Systolic pressure variation, defined as the difference between the maximum and minimum values during a breathing cycle can be divided into the so-called delta up, the increase; and delta down, the decrease of pressure. These parameters are affected by several factors besides volume status, the type of respiration (spontaneous or mechanical), the lung and chest wall compliance and the cardiac function, and the type and dose of sedation. The pressure changes are regarded as the result of changing intrathoracic pressure induced variations in venous return, pulmonary circulation, right and left ventricular afterload, and finally stroke volume (Toska and Eriksen, 1993; Rooke, 1995).

Why are blood pressure fluctuations more prominent in hypovolemia? On one hand, it is believed that respiration-related variations in venous return and stroke volume can be increased in hypovolemia. In addition, as Taylor demonstrated, the area of the ascending aorta decreases during non-hypotensive hypovolemia. This study suggests that small reduction in blood volume reduce aortic baroreceptor area, and arterial baroreflexes can be activated without detectable arterial pressure alterations (Taylor et al., 1995). Barbieri argues that the decreased arterial capacitance itself can result in larger pressure variation (Barbieri et al., 2002).

Does only ventilation-related blood pressure fluctuation increase in hypovolemia? Is it accompanied also with changed heart rate variability? In a study of simulated gravity McKenzie found significant increase in systolic and diastolic pressure LF power, and also an even greater significant increase in HF powers. The RRI power showed no consistent change (McKenzie, 1993). Fortrat found increased high frequency blood pressure variability, and no alteration of RRI variability during blood donation (Fortrat et al., 1998). During upright tilt Cooke found that SAP and DAP spectral power increased in LF and in HF, while the RRI LF power was unaffected and the RRI HF power decreased progressively with the tilt angle (Cooke et al., 1999). In our study the HF blood pressure variability increased significantly, although LF SAP fluctuations also tended to increase. The RRI power changes were different

in LF and HF. While increased HF fluctuations of blood pressure can be the manifestation of greater mechanical effect of greater stroke volume variation, the LF fluctuations can reflect increased sympathetic activity. The inconsistency of the data in literature regarding RRI LF power requires further explanation. It is possible, that the trigger of baroreflex (blood pressure fluctuation) increased, but the gain in heart rate decreased at the same time, and the net effect depends on the magnitude of these opposite influences. In our study this resulted in increased RRI variation in the low frequency band. The decrease in high frequency power of RR intervals, i.e. the decreased respiratory sinus arrhythmia, can be the effect of sympathetic activation and concomitant vagal withdrawal. So the increased SAP and DAP respiratory fluctuations also can result from the attenuated buffering effect of heart rate (Cooke et al., 1999).

4.4.2. Cardiac vagal baroreflex gain

Data of the influence of volume loading and unloading on human cardiac vagal baroreflex gain are conflicting. The central volume changes can be induced not only by direct blood withdrawal or volume loading, but by different posture, head-up tilt position, by lower body negative pressure or by simulated gravity, so we can parallel our result with such investigations. In an early study Takeshita provoked central venous pressure changes by lower body negative pressure (LBNP) and by leg and lower trunk elevation. The arterial baroreflex gain measured by the phenylephrine and neck suction methods was not changed (Takeshita et al., 1979). Similarly, Eiken found that LBNP induced reduction of central venous pressure did not influence baroreflex control of heart rate, assessed by neck chamber technique over the entire arterial pressure RRI relation (Eiken et al., 1994). In a similar study to ours, Fortrat found that the spontaneous cardiac vagal baroreflex gain did not change after 480 ml blood donation. They concluded that the slight sympathetic activation due to blood withdrawal elicited rapid resetting of the baroreflex (Fortrat et al., 1998)]. Steptoe and Vögele observed in their study of postural change from sitting to standing the reduction of the slope of spontaneous sequences (Steptoe and Vögele, 1990). In another study, the spontaneous cardiac baroreflex gain decreased after active standing and head-up tilt position, while the number of sequences increased signing greater baroreflex engagement (Bahjaoui-Bouhaddi et al., 1998).

Cooke also demonstrated that baroreflex gain decreased significantly at higher degrees of head-up tilt position (Cooke et al., 1999). In a recent trial Barbieri concluded that short-term cardiovascular control of heart rate appears to be optimized at mild hypervolemia. They manipulated central blood volume by LBNP and by leg elevation and volume loading. They found that the baroreflex gain decreased with volume unloading (Barbieri et al., 2002).

Why do cardiac vagal baroreflex gain decrease with preload reduction? There are different theoretical solutions for this problem. There can be a cardiopulmonary arterial baroreflex interaction. According to this hypothesis, the unloading of the cardiopulmonary receptors results in sympathetic activation. This sympathetic activation can modify centrally and peripherally the vagal responses. However, in contrast to animals, it is difficult to prove cardiopulmonary-arterial baroreflex interactions in humans, because the influence of the aortic baroreceptors cannot be excluded. As mentioned above, aortic baroreceptors can be activated by alterations in aortic blood volume causing changes in aortic baroreceptor area, even without measurable changes in blood pressure (Taylor et al., 1995). According to Cooke, there is also the possibility that the operational range shifts to the threshold region of the arterial pressure RR interval relationship during central hypovolemia (Cooke et al., 1999).

From our results we cannot answer the question, that if the cardiac vagal baroreflex gain deceases with volume unloading, why it was not reflected by all baroreflex estimates. There are data, that the responses to increasing and decreasing blood pressure are not the same (Pickering et al., 1972). This can explain that though the direction was the same, the magnitude of changes of up sequence and down sequence BRS was not identical.

The results of spectral baroreflex gain are more difficult to explain. The phase angles can give further insight into the problem. The phase shift we found in low frequency band is almost exactly the same, what Cooke found (Cooke et al., 1999). It was in our study -58±24° before and -54±26° after blood withdrawal. The phase angle between two waves at a given frequency could be translated into time delays. Baroreflex mediated time delays are determined by response times, conduction velocities and neurotransmitter kinetics, and therefore are fairly stable in different test conditions. These angles strongly support baroreflex relationship between SAP and RR interval in LF (Cooke et al., 1999; Cevese et al., 2001).

The relationship of blood pressure and RR interval fluctuations in the high frequency band could be assessed by taking several factors into consideration. The presence of a spectral

peak in both signals with identical central frequency may imply a cause-effect relationship. Such a relationship seems to be indicated by the high level of coherence between signals. The intertwined peaks nevertheless do exist following baroreflex denervation, and the level of coherence remains high (Di Rienzo et al., 1997). In our study the phase angles in HF were variable, unstable and basically incompatible with arterial baroreflex time delays. According to the phase angles, SAP and RRI changes happened nearly at the same time, suggesting that the spectral peaks should therefore be related to a third factor, which is respiration itself. Non-baroreflex mechanisms, such as pulmonary stretch receptor reflexes could be operational (Taha et al., 1995). After all, it was surprising, that HF alpha index behaved as it is expected from a baroreflex gain, it decreased. A recent study of Barbieri demonstrated that preload manipulations in humans bring on parallel modulations in baroreflex gain and respiratory sinus arrhythmia (Barbieri et al., 2002). This parallelism sufficiently explains the observed phenomenon. In the opposite, LF alpha index, which is regarded as a measure of baroreflex gain, even increased in 17 subjects, resulting in overall not significant change in average.

5. Summary

Arterial baroreflex control of heart rate and blood pressure is essential for maintaining adequate circulation. Furthermore, abnormal cardiovascular autonomic regulation is associated with worse prognosis in certain diseases, like after myocardial infarction and in heart failure. Unfortunately, in men we cannot measure directly the activity of baroreceptors, nor the activity of the sympathetic and parasympathetic nervous system. However, we can assess several surrogate parameters, some of which are widely used in clinical practise. Traditionally, we describe the time and frequency domain parameters of heart rate and blood pressure variability; we can quantify the rapid (vagal) changes in RR interval in relation to blood pressure changes (the cardiac vagal baroreflex gain) and sympathetic efferent responses (muscle sympathetic nerve activity). For cardiac vagal baroreflex gain determination there are several methods available, but though the calculated baroreflex indices are related, their absolute values might be different. In addition, nor the influence of study conditions, nor the normal values, nor the reproducibility of these parameters are clearly defined.

In our studies we investigated the clinical applicability and reproducibility of the most commonly used heart rate and blood pressure variability parameters and non-invasive baroreflex gain indices. In addition, we assessed how does respiration and hypovolemia influence these parameters.

In summary, in these groups of healthy individuals both non-invasive methods (linear spontaneous sequence method and cross spectral analysis) proved to be well applicable for BRS determination. Our results indicate that linear and spectral baroreflex gain indices are related but are not identical. The baroreflex gain derived from the phase IV of the Valsalva manoeuvre though correlates with the other estimates, is not clinically reliable.

Regarding reproducibility of heart rate and blood pressure variability parameters and spontaneous baroreflex indices existing data are not convincing. Different methods are prone to different degrees of influence resulting from variations in test settings. According to our investigations respiratory rate has major influence on all of these parameters, especially in frequency domain. So standardized study conditions are necessary (but are not always enough) to be able to assess the impairment or improvement in autonomic regulation and to draw clinically relevant conclusions. Slow patterned breathing may increase the validity and applicability of these methods.

In our study of hypovolemia rapid volume loss resulted not only in sympathetic activation (that was reflected in increased systolic, diastolic and mean blood pressure variability), but was associated with vagal withdrawal. Blood pressure variability mainly increased in high frequency band that could have been related to the decreased buffering effect of heart rate. The decreased vagal responsiveness also resulted in smaller up sequence baroreflex gain. The time relationship between RR interval and systolic arterial pressure changes (that is reflected in spectral analysis in the phase angle between the two waveforms) remained fairly constant before and after volume loss in low frequency band, and was compatible with baroreflex mechanism. The phase angles in high frequency range did not support baroreflex mechanism, so the high frequency alpha index is not recommended to use for baroreflex gain estimation.

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8. Figures

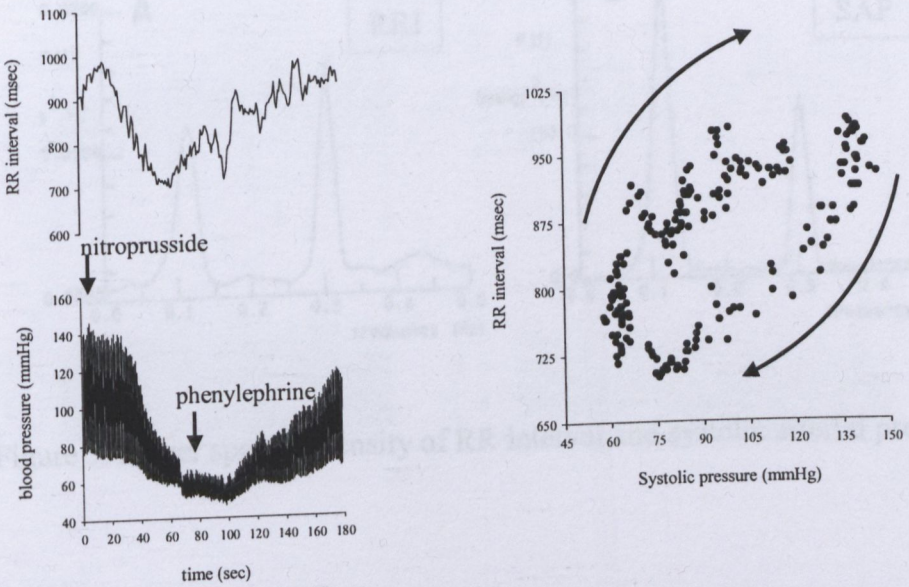

Figure 1. Pharmacological (Oxford method) for baroreflex gain determination

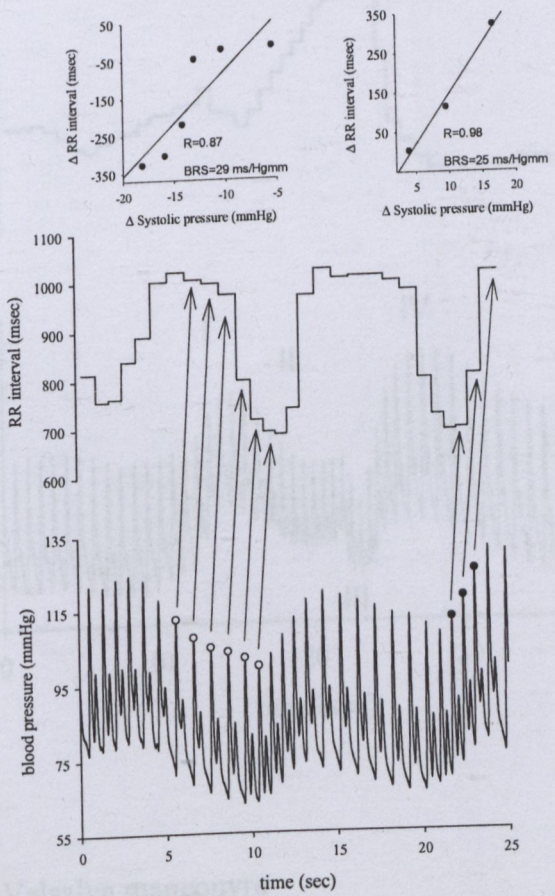

Figure 2. Spontaneous sequence method for baroreflex gain determination

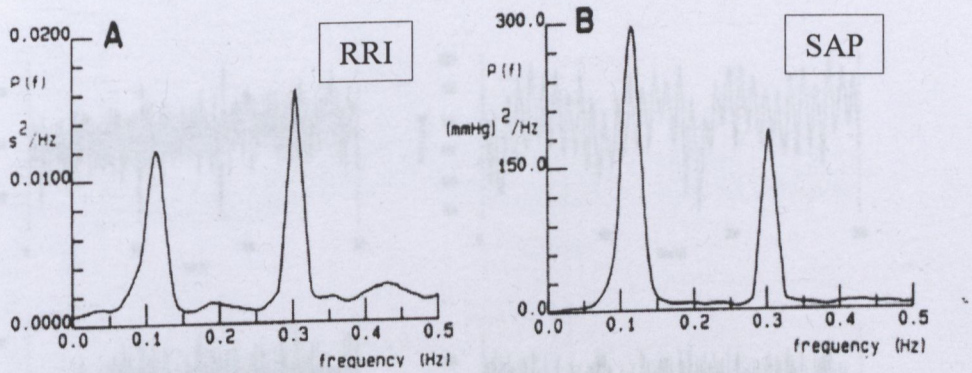

Figure 3. Power spectral density of RR interval and systolic arterial pressure

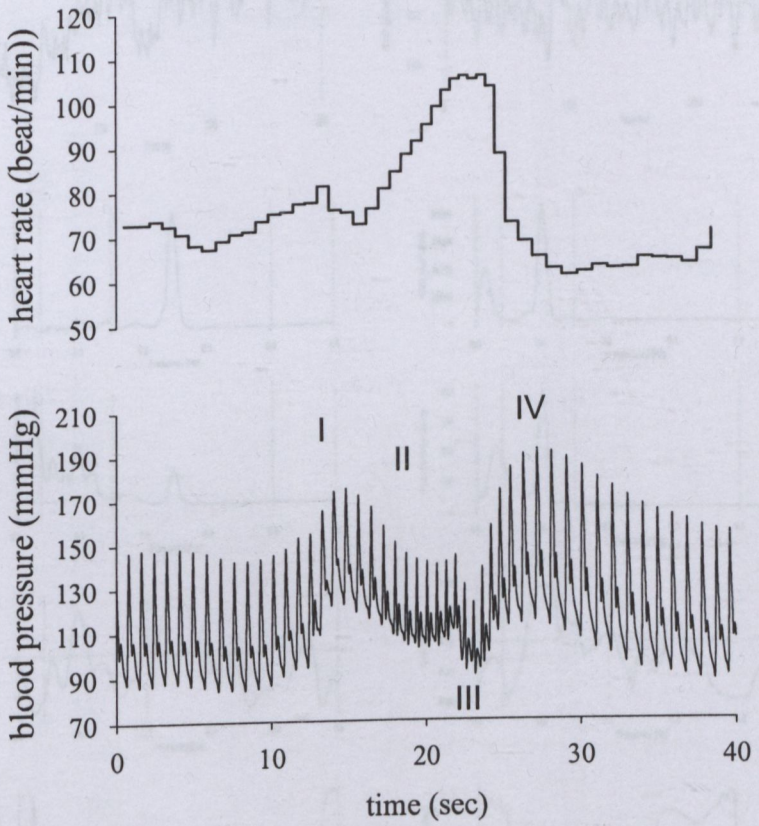

Figure 4. The Valsalva maneouvre

15/min respiratory rate

6/min respiratory rate

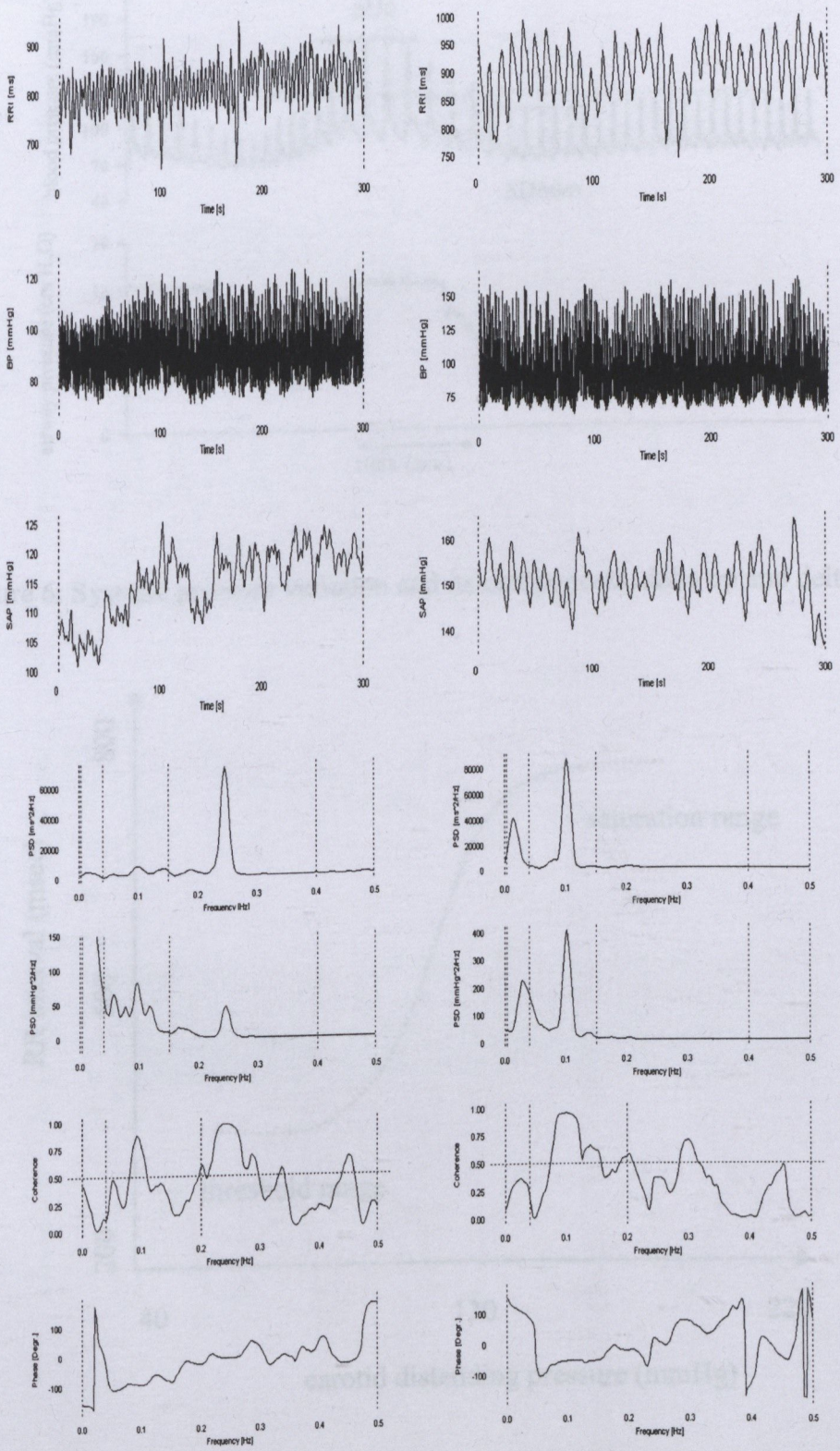

Figure 5. The effects of different respiratory rate on RR interval, blood pressure and on the frequency domain parameters of heart rate and blood pressure variability

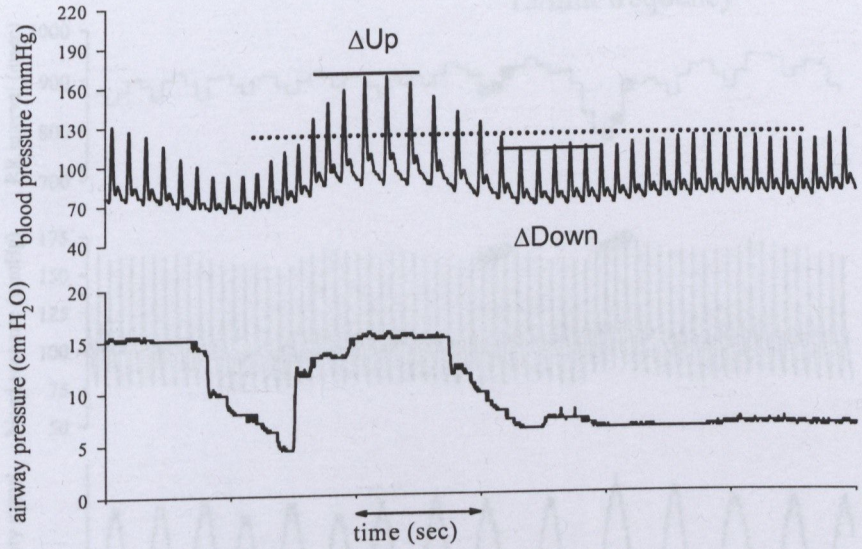

Figure 6. Systolic pressure variation and its components, delta up and delta down

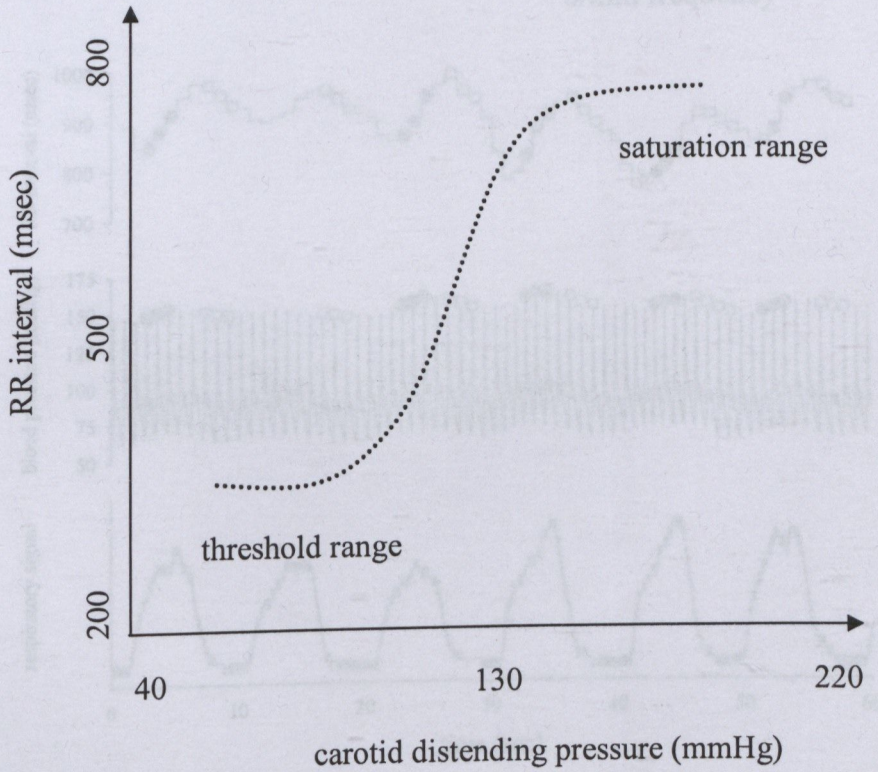

Figure 7. The characteristic sigmoid RR interval carotid distending pressure relation

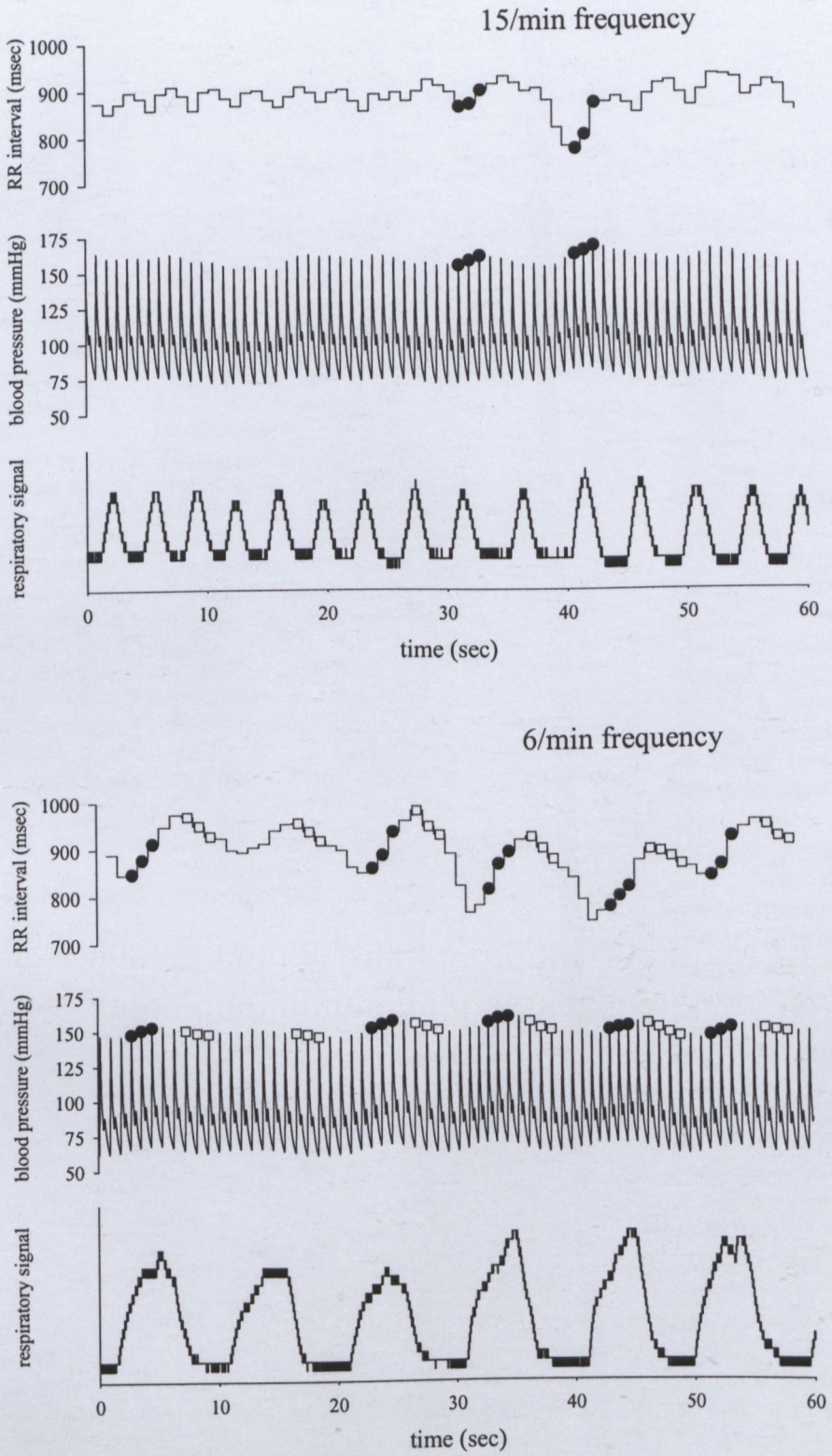

Figure 8. The effect of breathing frequency on the number of spontaneous sequences

8. Appendix

I.