Parasympathetic function during deep breathing in the general population: relation to coronary risk factors and normal range

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Objectives. To examine the association between the parasympathetic function assessed by deep breathing-induced heart rate variability (HRV) and coronary risk factors and to establish a reference range for deep breathing-induced HRV.

Design. Cross-sectional population-based study.

Setting. The municipality of Horsens, Denmark.

Subjects. One hundred and ninety-four individuals aged 40–67 years without diabetes, atrial fibrillation or a pacemaker randomly selected from the population.

Main outcome measures. During deep breathing at 6 respiratory cycles min⁻¹, an ECG was taken and in three consecutive cycles the longest R–R interval during expiration (E) and the shortest during inspiration (I) were selected. The mean ratio (E/I\textsubscript{ratio}) and the mean difference in instantaneous heart rate (E/I\textsubscript{diff}) were taken as expressions of the parasympathetic function.

Results. In multivariate analysis, E/I\textsubscript{diff} was reduced with increasing age (P < 0.0005) and left ventricular mass (P = 0.008), with ECG sign of probable previous myocardial infarction (P = 0.020) and the use of cardiac medication (P = 0.018) and positively correlated to heart rate (P = 0.030). The E/I\textsubscript{ratio} was diminished with increasing age (P = 0.001), left ventricular mass (P = 0.003), waist–hip ratio (P = 0.044), with ECG sign of probable previous myocardial infarction (P = 0.012) and use of cardiac medication (P = 0.020), but no association was found with heart rate (P = 0.92). In both E/I\textsubscript{diff} and E/I\textsubscript{ratio}, no correlation was found to lipids, blood pressure or alcohol consumption. In the group not on cardiac medication, without left ventricular hypertrophy or ECG signs of myocardial infarction, E/I\textsubscript{diff} and E/I\textsubscript{ratio} were still independently correlated to age and left ventricular mass. In this group, equations defining the age-corrected 5th percentile were calculated.

Conclusions. The parasympathetic function as assessed by deep breathing-induced HRV in the general population is reduced in older people, and in individuals on cardiac medication, with left ventricular hypertrophy or ECG signs of myocardial infarction. Even in healthy persons the parasympathetic function is inversely associated with age and left ventricular mass. Values of E/I\textsubscript{diff} above (4.39–0.033 \times \text{age})² and readings of E/I\textsubscript{ratio} above 1 + \exp (–1.12–0.0198 \times \text{age}) can be regarded as normal.

Keywords: ageing, autonomic nervous system, heart rate variability, humans, left ventricular mass, population.

Introduction

Reduced heart rate variability (HRV) is an independent marker of poor prognosis after an acute myocardial infarction (MI) [1] and in diabetes reduced HRV indicates the presence of cardiovascular autonomic neuropathy (CAN), which also implies a poor prognosis [2, 3]. However, assessing HRV from 24 h ECG recordings, whether in the time or frequency domain, is time-consuming and relies on expensive
Electronic equipment. Expressions of HRV may, nonetheless, be obtained by a more simple and less expensive technique.

Since the late 1970s, HRV measured with a ruler on an ECG obtained during spontaneous respiration has been known to reflect the prognosis after MI [4]. HRV during deep breathing (DB-HRV) is approximately doubled that during quiet breathing [5]; so, due to the larger variation in the R–R intervals, DB-HRV is probably a more precise measure, and recently DB-HRV has been shown to be an independent prognostic marker after MI [6].

In the examination of autonomic neuropathy in diabetes, DB-HRV constitutes one of an established battery of five tests [7]. It is often assumed that all five tests are necessary to diagnose CAN reliably, but no evidence exists to support this postulate. The deep breathing test is the most attractive of the five for several reasons. The parasympathetic function is reduced early in the development of CAN and HRV induced by deep breathing is almost exclusively mediated by the parasympathetic fibres, whereas the other tests are mediated in a more complex manner [8]. Moreover, measuring DB-HRV only takes a few minutes, and it can be carried out in any hospital setting as it only requires an electrocardiographic apparatus, a watch and a ruler. The method is thus convenient to use as a screening test for CAN.

Few reports concerning normal values of DB-HRV based on differently selected individuals are available [7, 9, 10]. The normal ranges in different age groups have been presented in tables, but today a mathematical expression for programming on a calculator or a computer would be more convenient. The association between DB-HRV and coronary risk factors has not been studied previously.

The purpose of this paper is to assess the associations between DB-HRV and established coronary risk factors in individuals randomly selected from the general population and to provide a reference range of DB-HRV presented as a simple mathematical expression.

**Methods**

**Subjects**

The individuals included in the study were examined when they participated as control persons in a survey on silent ischaemia carried out in the municipality of Horsens, Denmark [11]. From the Central Population Register, 194 individuals were randomly selected to match a diabetic sample with regard to age and gender. If one of the control persons refused to participate or was proven diabetic, another was randomly drawn.

**Deep breathing-induced heart rate variability**

The participants were instructed to breathe deeply at a frequency of 6 cycles min$^{-1}$ whilst sitting up. During the procedure a six-lead electrocardiogram (ECG) was obtained at 50 mm s$^{-1}$, and the beginning of each inspiration and expiration marked on the ECG with a pencil. The ECG was blinded by a random number before it was interpreted. In each respiratory cycle the longest R–R interval during expiration and the shortest during inspiration were selected using a pair of compasses, and the R–R intervals measured by a ruler to the nearest 0.5 mm. The ECGs were examined by two different persons, and if a reading differed by more than 0.5 mm the ECG was measured once more to reach agreement. The heart rates corresponding to these R–R intervals were calculated and the mean difference between the highest and lowest instantaneous heart rates in three consecutive respiratory cycles (E/Idiff) was used in the analysis. The DB-HRV was also expressed as the ratio between the longest and the shortest R–R interval in each respiratory cycle averaged over three consecutive respiratory cycles for the analysis (E/I_{max}).

**Electrocardiography at rest**

A 12-lead ECG taken at rest was blindly interpreted according to the Minnesota code [12] by two experienced technicians and the presence of code 1.1, 1.2 (significant Q-waves), or 7.1 (left bundle branch block) was taken as a sign of a probable previous myocardial infarction [13] (ECG-MI).

**History**

All participants were asked if they, or any of their parents, had ever had a myocardial infarction, about leisure exercise, smoking habits and angina pectoris (Rose questionnaire [14]). From precoded forms the questions were read to each participant. A history of previous myocardial infarction was only accepted for the analysis, if verified by hospital records.
Biochemical analyses

A fasting blood sample was taken at 08.00 h. Plasma total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, lipoprotein (a), and apolipoprotein A1 and B, were measured according to hospital routine methods.

Echocardiography

Echocardiography was carried out using standard two-dimensional projections and motion mode with a Toshiba SSA-140 A machine and a 2.5-MHz phased array transducer. Transmitral flow velocities were recorded by pulsed wave Doppler, the sampling depth adjusted to the maximal excursion of the anterior mitral leaflet. With continuous wave Doppler, the beam going through the left ventricle outflow tract as well as the anterior mitral leaflet, a trace depicting both the left ventricle outflow and inflow was recorded. Isovolumic relaxation time (IRT) was registered from the end of left ventricle outflow to the beginning of next inflow. In case of a homogenous contraction pattern of the left ventricle, the ejection fraction was calculated from the motion mode readings [15], but if regional dyskinesia was present, the ejection fraction was assessed from wall motion index using the nine segment model [16]. From the diastolic motion mode readings, the mass of the left ventricle (LVM) was calculated [17]:

$$LVM = 0.8\{1.04 [(\text{interventricular septum} + \text{left ventricular diameter} + \text{posterior wall})^3 - (\text{left ventricular diameter})^3]\} + 0.6 \text{ g}$$

Left ventricular hypertrophy was considered present if the left ventricular mass index exceeded 100 g m$^{-2}$ in women and 131 g m$^{-2}$ in men [18]. All recordings were carried out in the expiration phase, and the mean of two readings was used in the calculations.

Blood pressure

To avoid observer-induced bias all manually measured blood pressure values were recorded with a Hawksley random zero manometer [19]. The blood pressure was measured twice or more until the difference between two consecutive readings was $\leq 4$ mmHg; the mean of the two was used in the calculations. After at least 10 min in the supine position, resting blood pressure was measured. An ambulatory blood pressure recording (TM2420) with auscultatory readings every 30 min for 24 h was carried out. Hypertension was defined as a resting blood pressure $\geq 140/90$ mmHg and/or the use of antihypertensive medication.

Ethics

The study complies with the declaration of Helsinki II [20], and the study protocol was approved by the local ethics committee. Written informed consent was obtained from each participant after verbal and written information was given. The results of each individual examination were forwarded to the participant as well as the general practitioner, and in case of any abnormal results, the participant was offered a consultation at the hospital.

Statistics

As the distributions of the majority of the continuous variables were positively skewed, the medians were given and the variations expressed as percentiles. Differences between groups were tested using the Mann–Whitney test and coefficients of correlation given as Spearman rho. The Kolmogorov–Smirnov test was used to compare distributions of the E/I_diff and E/I_ratio with the Gaussian distribution. After transformation towards normal distribution, the independent correlations between E/I_diff and E/I_ratio to different coronary risk factors were assessed by the multiple regression analysis. If a bivariate test between a coronary risk factor and the E/I_diff or E/I_ratio showed a $P$-value below 0.10, the risk factor was included in the multivariate analysis. Using the backward elimination principle, variables with insignificant relation to E/I_diff or E/I_ratio were excluded one by one, the least significant first. A risk of a type-one error of 0.05 was accepted.

Results

Basic characteristics

One hundred and ninety-four persons were included. The age range was 40–67 years and 56% were males. The prevalence of cardiovascular disease was low (Table 1). Twenty-one of the participants (11%) took some kind of cardiac medication (ACE inhibitors (5), beta-blockers (6), calcium blockers (2), diuretics (16), nitrates (1); nobody took digoxin or antiarrrhythmics). No-one had diabetes mellitus. Two cases had to be excluded due to poor co-operation: the remaining 192 completed the examination. No-
one had atrial fibrillation, bundle branch block or a pacemaker.

The mean of the shortest R–R interval during inspiration was 37.4 mm (SD = 6.1 mm), corresponding to an instantaneous heart rate of 80.2 min⁻¹, and the mean of the longest R–R interval in the expiration was 48.8 mm (SD = 8.5 mm), corresponding to a heart rate of 61.5 min⁻¹. The mean E/Iₗ was 18.8 min⁻¹ and the mean E/Iₘ was 1.31. To assess the influence of the error introduced by the use of a ruler, we calculated the maximum possible deviation from the true mean E/Iₗ that could be caused by a 0.5 mm reading error. After subtracting 0.5 mm from each of the short R–R intervals measured and adding 0.5 mm to each of the long R–R intervals, the mean E/Iₗ was elevated to 20.7 min⁻¹, corresponding to an increase of 1.85 min⁻¹ (9.8%). Correspondingly, the maximum possible lowering of the E/Iₗ that could be caused by the use of a ruler was calculated to 1.83 min⁻¹ (9.7%). In the same way the maximum positive deviation in the E/I ratio that could be caused by the reading error was calculated to be 0.032 (2.5%) and the maximum negative deviation to be 0.031 (2.4%).

Deep breathing-induced heart rate difference

In bivariate analysis the difference in instantaneous heart rate induced by deep breathing, E/Iₗ correlated with high significance to age, but also to LVM, heart rate at rest, IRT, the presence of ECG-MI and whether or not any cardiac medication was taken (Tables 1 and 2). The distribution of E/Iₗ was skewed to the right (goodness-of-fit test against the normal distribution: P = 0.039) and was square-root transformed (goodness-of-fit test against the normal distribution: P = 0.57) for further analysis. Coronary risk factors with at least a borderline (P < 0.10) correlation to E/Iₗ (Tables 1 and 2) were chosen for multivariate analysis. E/Iₗ was independently and inversely associated with age (P = 0.0005), LVM (P = 0.008), ECG-MI (P = 0.020), and cardiac medication (P = 0.018) and positively correlated to heart rate (P = 0.030). Body mass index (P = 0.79), IRT (P = 0.76) and smoking (P = 0.98) were excluded from the model.

In order to obtain values from individuals free of cardiovascular disease interfering with E/Iₗ, persons on cardiac medication, with left ventricular hypertrophy or ECG-MI were omitted. Regression of E/Iₗ on age was carried out in the remaining 158 participants (a = 5.99, b = -0.033, s.e.e. = 0.98, r = 0.22, P = 0.005). A line parallel to the regression line corresponding to the 5th percentile was computed:

5th percentile (E/Iₗ) = 4.39 - 0.033 × age (years)

To obtain a reference limit of the non-transformed readings (Fig. 1A) both sides of the equation were squared:

5th percentile (E/Iₗ) = (4.39 - 0.033 × age (years))^2

In the group without cardiac medication, left ventricular hypertrophy or ECG-MI, E/Iₗ was still significantly inversely related to LVM (P = 0.009) and positively correlated to heart rate (P = 0.008) in a regression model controlling for age (P < 0.0005). No significant associations were uncovered when this model was subsequently enhanced with the mean
ambulatory systolic \((P = 0.27)\) and diastolic \((P = 0.75)\) blood pressures, one at a time.

**Deep breathing-induced heart rate ratio**

The \(E/I_{\text{ratio}}\) was significantly related to age \((P < 0.0005)\), IRT \((P = 0.022)\), cardiac medication \((P = 0.007)\) and ECG-MI \((P = 0.005)\) in a bivariate analysis (Tables 1 and 2). The \(E/I_{\text{ratio}}\) also had a right skewed distribution (goodness-of-fit test against the normal distribution: \(P = 0.036\)), and the logarithm of the ratio minus 1 was taken for further analysis (goodness-of-fit of \(\ln(E/I_{\text{ratio}} - 1)\) against the normal distribution: \(P = 0.87\)). Again, coronary risk factors with at least a borderline \((P < 0.10)\) correlation to \(E/I_{\text{ratio}}\) (Tables 1 and 2) were selected for multivariate analysis. The \(E/I_{\text{ratio}}\) was independently and inversely associated with age \((P = 0.001)\), LVM \((P = 0.003)\),
ECG-MI (P = 0.012) and cardiac medication (P = 0.020), IRT (P = 0.91) was excluded from the model. Individuals taking cardiac medication, with left ventricular hypertrophy or with ECG-MI were then omitted and regression of the E/I ratio on age was carried out in the remaining 158 participants (a = −0.227, b = −0.0198, s.e.e. = 0.514, r = 0.26, P = 0.001). Again, a mathematical expression of the 5th percentile was computed:

\[
\text{5th percentile (ln(E/I ratio − 1))} = -1.12 - 0.0198 \times \text{age (years)}
\]

To obtain a reference limit of the non-transformed measurements (Fig. 1B), the exponential function was taken and 1 was added to both sides of the equation:

\[
\text{5th percentile (E/I ratio)} = 1 + \exp(-1.12 - 0.0198 \times \text{age (years)})
\]

In the group without cardiac medication, left ventricular hypertrophy or ECG-MI, the E/I ratio was still significantly and inversely related to LVM (P = 0.033) in a regression model controlling for age (P < 0.0005). No significant associations were revealed when the mean ambulatory systolic (P = 0.18) and diastolic (P = 0.66) blood pressures were added to this model one at a time.

**Discussion**

Deep breathing-induced HRV could be measured in 99% of this group randomly selected from the general population. The examination is thus both easy to perform and applicable in the majority of individuals. The error introduced by the use of a ruler could at worst cause a deviation from the mean E/I ratio of 10% and from the mean E/I diff of 2.5%. The error caused by the use of a ruler does not, therefore, seem to be a major problem.

**Age**

Age was strongly associated with deep breathing-induced HRV, and a significant degree of misclassification may be introduced if a reference range is used which does not take age into account. Reference limits often used in the literature specify that an E/I diff ≤ 10 min⁻¹ is abnormal and that values ≥ 15 min⁻¹ are normal, leaving a borderline zone between 10 and 15 min⁻¹ [7]. If persons in this study with an E/I diff < 15 were classified as non-normals, 71 (37%) of the participants would have been registered as non-normal, and 62 (34%) would then have been misclassified according to our reference limits for E/I ratio. If the limit of E/I ratio ≤ 10 min⁻¹ signifying a definite abnormal HRV had been used, 28 (15%) of these individuals randomly selected from the general population would have been categorized as having reduced HRV and 19 (10%) would have been misclassified according to our reference limit. Therefore, although the effect of age only predicts 6.3% of the total variation, age must be taken into account when reference limits are considered.

**Myocardial infarction**

The analysis indicated that electrocardiographic signs of previous MI, which include asymptomatic infarctions, had a stronger influence on HRV than previous clinically manifest MIs. This could seem strange at first, but the finding may be explained by differences in infarction size as the clinical MIs included non-Q infarctions, but large Q-waves were present when ECG-MIs were registered.

**Comparison of deep breathing-induced heart rate difference and heart rate ratio**

In the present study, an independent association was shown between the E/I ratio and heart rate at rest. In some previous investigations carried out on healthy persons, this relation was also found [21–23], but in other studies it could not be demonstrated [7, 24, 25]. The E/I ratio, on the other hand, was not correlated with heart rate in our material. This expression of deep breathing-induced HRV has been studied less extensively. A significant relation to heart rate has been reported by some authors [23, 24] but not found by others [26].

In animal experiments it has been shown that an increase in the parasympathetic tone decreases heart rate in a hyperbolic fashion [27]. Accordingly, due to purely mathematical reasons, measures of vagal modulations given as differences in instantaneous heart rate depend on the initial heart rate. A given change in the parasympathetic tone from a low level (high heart rate) results in a larger difference in the instantaneous heart rate compared with a change starting from a higher level (lower heart rate). The length of the R–R interval, on the other hand, is related to vagal tone in a linear fashion [27]. Thus, in healthy individuals it should be expected that E/I diff
but not E/I\text{ratio}, is associated with heart rate. The relations of heart rate to E/I\text{diff} and E/I\text{ratio} found in our study are consistent with this theory. In individuals with an elevated heart rate due to autonomic neuropathy, the measured reduction in HRV will be less pronounced if expressed by E/I\text{diff} and the presence of autonomic neuropathy could be missed. The E/I\text{ratio} may therefore be the better expression to use.

**Left ventricular mass**

HRV, whether expressed as E/I\text{diff} or E/I\text{ratio}, was inversely related to LVM. This relation has previously been reported in studies of diabetics with neuropathy [28] and in individuals with aortic stenosis [29] or hypertension [30, 31], but reduced HRV has not previously been linked to LVM in healthy human beings. Increased LVM implies an adverse prognosis with regard to mortality [32] and especially sudden death [33], and the same prognostic implications apply to reduced HRV [34, 35]. It has been shown that experimental stimulation of the adrenergic receptors in a dose not affecting any haemodynamic parameters can induce myocardial hypertrophy [36], and it has recently been shown that LVM is coupled to cardiac noradrenaline release in a small normotensive group [37]. It is thus possible that increased sympathetic activity per se is a causal factor behind reduced HRV, increased LVM and sudden death.

**Limitations of the study**

The generalization of our results is limited by the age range of the study group (40–67 years). In order to find out how our reference range formulas worked outside this age range, we compared the calculated reference limits with other materials. Smith [26] examined 174 healthy persons in the age range 16–89 years. The 5th percentile value for the E/I\text{ratio} using our formula was calculated from the midpoint of the age groups defined by Smith. The E/I\text{ratio} was (this study/Smith): for 18 years, 1.23/1.27; 28 years, 1.19/1.22; 38 years, 1.15/1.17; 48 years, 1.13/1.13; 58 years, 1.10/1.10; 68 years, 1.08/1.08; 78 years, 1.07/1.06. O’Brien et al. [38] assessed the E/I\text{diff} in 310 healthy persons aged 18–85 years, and tabulated the reference limit in age groups. The reference limit (5th percentile) of E/I\text{diff} was (this study/O’Brien et al.): for 20 years, 4/4 min\textsuperscript{-1}; 30 years, 12/11 min\textsuperscript{-1}; 40 years, 9/9 min\textsuperscript{-1}; 50 years, 8/7 min\textsuperscript{-1}; 60 years, 6/5 min\textsuperscript{-1}; 70 years, 4/4 min\textsuperscript{-1}; and 75 years, 4/3 min\textsuperscript{-1}. Included in O’Brien et al.’s material were factory employees, hospital staff, healthy outpatients and volunteers from day centres for the elderly. In Smith’s and O’Brien et al.’s material a single deep breath was applied, whilst we used three consecutive deep breaths, which has a greater reproducibility [39]. In spite of differences in materials and methods, the 5th percentiles calculated from our formulas are practically identical to the values tabulated in both studies. Thus, although our formulas, strictly speaking, are valid only for the age range 40–67 years, they may be applicable in a wider range of age.

**Conclusions**

Measuring HRV by the deep breathing method is easy and can be carried out in 99% of the general population. Values of E/I\text{diff} above (4.39 – 0.033 \times \text{age (years)})\textsuperscript{2} and of E/I\text{ratio} above 1 + \exp(-1.12 – 0.0198 \times \text{age (years)}) can be regarded as normal. HRV induced by deep breathing in the general population is reduced in individuals on cardiac medication, with left ventricular hypertrophy or ECG signs of previous MI. HRV is inversely associated with age and left ventricular mass, even in healthy individuals. Future investigations will clarify whether this simple examination of HRV or the more sophisticated expressions of HRV based on 24 h ECG recordings is the better predictor of prognosis.

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**References**
