

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

O. MAY, H. ARILDSEN & M. MØLLER

From the Department of Cardiology, Odense University Hospital, and Department of Medicine, Horsens Hospital, Denmark

**Abstract.** May O, Arildsen H, Møller M (Odense University Hospital, Odense and Horsens Hospital, Horsens, Denmark). Parasympathetic function during deep breathing in the general population: relation to coronary risk factors and normal range. *J Intern Med* 1999; 245: 287–94.

**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  $\text{min}^{-1}$ , 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 ( $E/I_{\text{ratio}}$ ) and the mean difference in instantaneous heart rate ( $E/I_{\text{diff}}$ ) were taken as expressions of the parasympathetic function.

**Results.** In multivariate analysis,  $E/I_{\text{diff}}$  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  $E/I_{\text{ratio}}$  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  $E/I_{\text{diff}}$  and  $E/I_{\text{ratio}}$ , no correlation was found to lipids, blood pressure or alcohol consumption. In the group not on cardiac medication, without left ventricular hypertrophy or ECG sign of probable myocardial infarction,  $E/I_{\text{diff}}$  and  $E/I_{\text{ratio}}$  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  $E/I_{\text{diff}}$  above  $(4.39 - 0.033 \times \text{age})^2$  and readings of  $E/I_{\text{ratio}}$  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 breath deeply at a frequency of 6 cycles  $\text{min}^{-1}$  whilst sitting up. During the procedure a six-lead electrocardiogram (ECG) was obtained at 50  $\text{mm s}^{-1}$ , 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 ( $E/I_{\text{diff}}$ ) 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 ( $E/I_{\text{ratio}}$ ).

### *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]:

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

Left ventricular hypertrophy was considered present if the left ventricular mass index exceeded  $100 \text{ g m}^{-2}$  in women and  $131 \text{ g m}^{-2}$  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  $E/I_{\text{diff}}$  and  $E/I_{\text{ratio}}$  with the Gaussian distribution. After transformation towards normal distribution, the independent correlations between  $E/I_{\text{diff}}$  and  $E/I_{\text{ratio}}$  to different coronary risk factors were assessed by the multiple regression analysis. If a bivariate test between a coronary risk factor and the  $E/I_{\text{diff}}$  or  $E/I_{\text{ratio}}$  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  $E/I_{\text{diff}}$  or  $E/I_{\text{ratio}}$  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 antiarrhythmics). No-one had diabetes mellitus. Two cases had to be excluded due to poor co-operation; the remaining 192 completed the examination. No-

**Table 1** Dichotomous coronary risk factors, prevalence and correlation to deep breathing-induced heart rate difference ( $E/I_{\text{diff}}$ ) and heart rate ratio ( $E/I_{\text{ratio}}$ )

	Prevalence (%)	Median $E/I_{\text{diff}}$ ( $\text{min}^{-1}$ )		Median $E/I_{\text{ratio}}$	
		Factor present/absent	<i>P</i>	Factor present/absent	<i>P</i>
Male gender	56	17.5/16.5	0.633	1.30/1.24	0.114
Currently smoking	50	15.9/18.4	0.096	1.26/1.31	0.135
Leisure exercise $\leq$ once a week	21	16.6/17.9	0.753	1.27/1.29	0.770
Hypertension	29	17.4/16.6	0.511	1.26/1.28	0.966
Angina pectoris	6	15.0/16.8	0.378	1.22/1.28	0.399
On cardiac medication	11	17.0/14.3	0.030	1.22/1.30	0.007
Previous MI	3	11.1/16.8	0.575	1.19/1.28	0.679
MI in a parent	16	18.8/16.6	0.115	1.32/1.27	0.204
ECG sign of previous MI	3	7.2/16.9	0.004	1.09/1.28	0.005

MI, myocardial infarction.

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  $\text{min}^{-1}$ , 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  $\text{min}^{-1}$ . The mean  $E/I_{\text{diff}}$  was 18.8  $\text{min}^{-1}$  and the mean  $E/I_{\text{ratio}}$  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  $E/I_{\text{diff}}$  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  $E/I_{\text{diff}}$  was elevated to 20.7  $\text{min}^{-1}$ , corresponding to an increase of 1.85  $\text{min}^{-1}$  (9.8%). Correspondingly, the maximum possible lowering of the  $E/I_{\text{diff}}$  that could be caused by the use of a ruler was calculated to 1.83  $\text{min}^{-1}$  (9.7%). In the same way the maximum positive deviation in the  $E/I_{\text{ratio}}$  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,  $E/I_{\text{diff}}$ , 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  $E/I_{\text{diff}}$  was skewed to the right (goodness-of-fit test against the normal distribution:  $P = 0.039$ ) and was square-root trans-

formed (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  $E/I_{\text{diff}}$  (Tables 1 and 2) were chosen for multivariate analysis.  $E/I_{\text{diff}}$  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  $E/I_{\text{diff}}$ , persons on cardiac medication, with left ventricular hypertrophy or ECG-MI were omitted. Regression of  $E/I_{\text{diff}}$  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:

$$\text{5th percentile } (\sqrt{E/I_{\text{diff}}}) = 4.39 - 0.033 \times \text{age (years)}$$

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

$$\begin{aligned} \text{5th percentile } (E/I_{\text{diff}}) = \\ (4.39 - 0.033 \times \text{age (years)})^2 \end{aligned}$$

In the group without cardiac medication, left ventricular hypertrophy or ECG-MI,  $E/I_{\text{diff}}$  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

**Table 2** Continuous coronary risk factors, median (2.5th and 97.5th percentile) and correlation to deep breathing-induced heart rate difference ( $E/I_{diff}$ ) and heart rate ratio ( $E/I_{ratio}$ )

	Median (2.5th, 97.5th percentile)	$E/I_{diff}$		$E/I_{ratio}$	
		Spearman <i>r</i>	<i>P</i>	Spearman <i>r</i>	<i>P</i>
Age (years)	51 (41, 65)	-0.231	0.001	-0.253	< 0.0005
Body mass index ( $kg\ m^{-2}$ )	25.6 (18.9, 36.6)	-0.141	0.051	-0.099	0.172
Waist-hip ratio	0.97 (0.75, 1.10)	0.088	0.227	0.137	0.058
Alcohol (units $week^{-1}$ )	6 (0, 45)	0.073	0.316	0.101	0.162
Resting systolic BP (mmHg)	126 (101, 171)	0.022	0.757	-0.026	0.723
Resting diastolic BP (mmHg)	78 (61, 98)	0.040	0.579	-0.003	0.966
Ambulatory systolic BP (mmHg)	122 (96, 155)	0.053	0.469	0.049	0.172
Ambulatory diastolic BP (mmHg)	76 (62, 94)	-0.056	0.446	-0.064	0.377
Cholesterol ( $mmol\ L^{-1}$ )	6.2 (4.1, 9.2)	0.011	0.875	-0.005	0.950
HDL cholesterol ( $mmol\ L^{-1}$ )	1.5 (0.8, 2.7)	-0.018	0.810	-0.046	0.525
LDL cholesterol ( $mmol\ L^{-1}$ )	4.1 (2.1, 6.3)	0.072	0.329	0.066	0.369
Triglyceride ( $mmol\ L^{-1}$ )	1.16 (0.56, 3.77)	0.003	0.969	-0.002	0.981
Apolipoprotein A1 ( $g\ L^{-1}$ )	1.74 (1.27, 2.57)	0.006	0.934	-0.030	0.681
Apolipoprotein B ( $g\ L^{-1}$ )	1.37 (0.79, 2.04)	-0.006	0.935	-0.013	0.861
Lipoprotein (a) ( $g\ L^{-1}$ )	0.25 (0.11, 1.7)	-0.020	0.792	-0.022	0.768
Left ventricular mass (g)	147 (85, 265)	-0.145	0.048	-0.083	0.258
Left ventricular mass index ( $g\ m^{-2}$ )	80 (47, 134)	-0.178	0.015	-0.124	0.090
Heart rate at rest ( $min^{-1}$ )	67 (47, 91)	0.143	0.048	-0.076	0.295
Ejection fraction (%)	77 (60, 88)	0.038	0.603	0.009	0.899
Mitral septal separation (mm)	2.9 (0.6, 8.3)	-0.104	0.153	-0.116	0.109
Early/late peak ratio	1.30 (0.72, 2.77)	0.042	0.560	0.118	0.103
Isovolumic relaxation time (ms)	104 (74, 145)	-0.230	0.001	-0.166	0.022

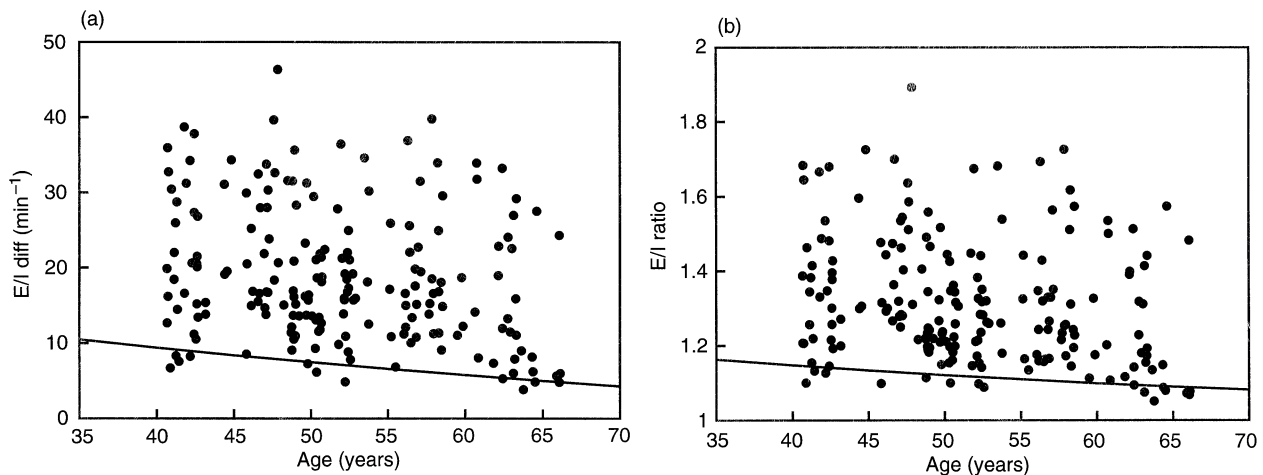
HDL, high-density lipoprotein; LDL, low-density lipoprotein; MI, myocardial infarction.

ambulatory systolic ( $P = 0.27$ ) and diastolic ( $P = 0.75$ ) blood pressures, one at a time.

*Deep breathing-induced heart rate ratio*

The  $E/I_{ratio}$  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  $E/I_{ratio}$  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  $\ln(E/I_{ratio} - 1)$  against the normal distribution:  $P = 0.87$ ). Again, coronary risk factors with at least a borderline ( $P < 0.10$ ) correlation to  $E/I_{ratio}$  (Tables 1 and 2) were selected for multivariate analysis. The  $E/I_{ratio}$  was independently and inversely associated with age ( $P = 0.001$ ), LVM ( $P = 0.003$ ),



**Fig. 1** Deep breathing-induced heart rate difference ( $E/I_{diff}$ ) (A) and heart rate ratio ( $E/I_{ratio}$ ) (B) plotted against age. Reference limits indicated.

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  $E/I_{\text{ratio}}$  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:

$$\begin{aligned} \text{5th percentile } (\ln(E/I_{\text{ratio}} - 1)) = \\ -1.12 - 0.0198 \times \text{age (years)} \end{aligned}$$

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:

$$\begin{aligned} \text{5th percentile } (E/I_{\text{ratio}}) = \\ 1 + \exp(-1.12 - 0.0198 \times \text{age (years)}) \end{aligned}$$

In the group without cardiac medication, left ventricular hypertrophy or ECG-MI, the  $E/I_{\text{ratio}}$  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  $E/I_{\text{diff}}$  of 10% and from the mean  $E/I_{\text{ratio}}$  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  $E/I_{\text{diff}} \leq 10 \text{ min}^{-1}$  is abnormal and that values  $\geq 15 \text{ min}^{-1}$  are normal, leaving a borderline zone between 10 and  $15 \text{ min}^{-1}$  [7]. If persons in this study with an  $E/I_{\text{diff}} < 15$  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  $E/I_{\text{diff}}$ . If the limit of  $E/I_{\text{diff}} \leq 10 \text{ min}^{-1}$  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  $E/I_{\text{diff}}$  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  $E/I_{\text{ratio}}$ , 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  $E/I_{\text{diff}}$ ,

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, 14/13  $\text{min}^{-1}$ ; 30 years, 12/11  $\text{min}^{-1}$ ; 40 years, 9/9  $\text{min}^{-1}$ ; 50 years, 8/7  $\text{min}^{-1}$ ; 60 years, 6/5  $\text{min}^{-1}$ ;

70 years, 4/4  $\text{min}^{-1}$ ; and 75 years, 4/3  $\text{min}^{-1}$ . 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  $(4.39 - 0.033 \times \text{age (years)})^2$  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.

## Acknowledgements

The study was supported by The 1991 Pharmacy Foundation, The Danish Heart Foundation, The Danish Medical Association Research Fund, The Vejle County Medical Research Foundation and Clinical Institute, Odense University, Denmark.

## References

- 1 Bigger JT, Fleiss JL, Steinman RC, Rolnitzky LM, Kleiger RE, Rottman JN. Frequency domain measures of heart period variability and mortality after myocardial infarction. *Circulation* 1992; **85**: 164–71.
- 2 Ewing DJ, Borse DQ, Bellavere F, Clarke BF. Cardiac autonomic neuropathy in diabetes: comparison of measures of R-R interval variation. *Diabetologia* 1981; **21**: 18–24.
- 3 Ewing DJ, Clarke BF. Autonomic neuropathy: its diagnosis and prognosis. *Baillieres Clin Endocrinol Metab* 1986; **15**: 855–88.

- 4 Wolf MM, Varigos GA, Hunt D, Sloman JG. Sinus arrhythmia in acute myocardial infarction. *Med J Austr* 1978; **2**: 52–3.
- 5 Wheeler T, Watkins PJ. Cardiac denervation in diabetes. *B M J* 1973; **4**: 584–6.
- 6 Katz A, Liberty IF, Porath A, Ovsyshcher I, Prystowsky EN. Simple bed side test of one minute heart rate variability during deep breathing predicts mortality after myocardial infarction. *Circulation* 1996; **94**: I27 (abstract).
- 7 Ewing DJ, Martyn CN, Young RJ, Clarke BF. The value of cardiovascular autonomic function tests: 10 years experience in diabetes. *Diabetes Care* 1985; **8**: 491–8.
- 8 Bellavere F. Heart rate variability in patients with diabetes and other noncardiological diseases. In: Malik M, Camm AJ, eds. *Heart Rate Variability*. Armonk, NY: Futura Publishing, Company, Inc., 1995; 507–16.
- 9 Hilsted J, Jensen SB. A simple test for autonomic neuropathy in juvenile diabetics. *Acta Med Scand* 1979; **205**: 385–7.
- 10 Wieling W, Brederode JF, Mv Rijk L, Gd Borst C, Dunning AJ. Reflex control of heart rate in normal subjects in relation to age: a data base for cardiac vagal neuropathy. *Diabetologia* 1982; **22**: 163–6.
- 11 May O, Arildsen H, Damsgaard EM, Mickley H. Prevalence and prediction of silent ischaemia in diabetes mellitus: a population-based study. *Cardiovasc Res* 1997; **34**: 241–7.
- 12 Rose GA, Blackburn H. *Cardiovascular Survey Methods*. Geneva: WHO, 1968.
- 13 Diabetes Drafting Group. Prevalence of small vessel and large vessel disease in diabetic patients from 14 centres. The World Health Organisation Multinational Study of Vascular Disease in Diabetics. *Diabetologia* 1985; **28**: 615–40.
- 14 Rose GA, Blackburn H, Gillum RE, Prineas RJ. *Cardiovascular Survey Methods*, 2nd edn. Geneva: WHO, 1982; 1–178.
- 15 Pompo JE, Troy BL, Russell RO. Left ventricular volumes and ejection fraction by echocardiography. *Circulation* 1971; **43**: 480–90.
- 16 Berning J, Nielsen JR, Launbjerg J, Fogh J, Mickley H, Andersen PE. Rapid estimation of left ventricular ejection fraction by echocardiography wall motion analysis. *Cardiology* 1992; **80**: 257–66.
- 17 Devereux RB. Evaluation of cardiac structure and function by echocardiography and other noninvasive techniques. In: Laragh JH, Brenner BM, eds. *Hypertension: Pathophysiology, Diagnosis and Management*. New York: Raven Press Ltd, 1990; 1479–92.
- 18 Savage DD, Garrison RJ, Kannel WB *et al.* The spectrum of left ventricular hypertrophy in a general population sample: The Framingham Study. *Circulation* 1987; **75**: 126–33.
- 19 Wright BM, Dore CF. A random-zero sphygmomanometer. *Lancet* 1970; **i**: 337–8.
- 20 World Medical Association, Inc. World Medical Association Declaration of Helsinki. Recommendations guiding physicians in biomedical research involving human subjects. *Cardiovasc Res* 1997; **35**: 2–3.
- 21 Smith SE, Smith SA. Heart rate variability in healthy subjects measured with a bedside computer-based technique. *Clin Sci* 1981; **61**: 379–83.
- 22 Beylot M, Haro M, Orgiazzi J, Noel G. Abnormalities of heart rate and arterial blood pressure regulation in diabetes mellitus. Relation with age, duration of diabetes and presence of peripheral neuropathy. *Diabetes Metab* 1983; **9**: 204–11.
- 23 Ziegler D, Laux G, Dannehl K *et al.* Assessment of cardiovascular autonomic function: age-related normal ranges and reproducibility of spectral analysis, vector analysis, and standard tests of heart rate variation and blood pressure responses. *Diabet Med* 1992; **9**: 166–75.
- 24 O'Brien IAD, O'Hare P, Corrall RJM. Heart rate variability in healthy subjects: Effects of age and the derivation of normal ranges for tests of autonomic function. *Br Heart J* 1986; **55**: 348–54.
- 25 Bennett T, Farquhar IK, Hosking DJ, Hampton JR. Assessment of methods for estimating autonomic nervous control of the heart in patients with diabetes mellitus. *Diabetes* 1978; **27**: 1167–74.
- 26 Smith SA. Reduced sinus arrhythmia in diabetic autonomic neuropathy: diagnostic value of an age-related normal range. *Br Med J* 1982; **285**(1): 1599–60.
- 27 Parker P, Celler BG, Potter EK. Vagal stimulation and cardiac slowing. *J Auton Nerv Syst* 1984; **11**: 226–31.
- 28 Gambardella S, Frontoni S, Spallone V *et al.* Increased left ventricular mass in normotensive diabetic patients with autonomic neuropathy. *Am J Hypertens* 1993; **6**: 97–102.
- 29 Mandawat MK, Wallbridge DR, Pringle SD *et al.* Heart rate variability in left ventricular hypertrophy. *Br Heart J* 1995; **73**: 139–44.
- 30 Kohara K, Hara-Nakamura N, Hiwada K. Left ventricular mass index negatively correlates with heart rate variability in essential hypertension. *Am J Hypertens* 1995; **8**: 183–8.
- 31 Piccirillo G, Munizzi MR, Fimognari FL, Marigliano V. Heart rate variability in hypertensive subjects. *Int J Cardiol* 1996; **53**: 291–8.
- 32 Kannel WB, Abbott RD. A prognostic comparison of asymptomatic left ventricular hypertrophy and unrecognized myocardial infarction: the Framingham study. *Am Heart J* 1986; **111**: 391–7.
- 33 Kannel WB, Gordon T, Offutt D. Left ventricular hypertrophy by electrocardiogram. Prevalence, incidence, and mortality in the Framingham study. *Ann Intern Med* 1969; **71**: 89–105.
- 34 Kleiger RE, Miller JP, Bigger JT, Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol* 1987; **59**: 256–62.
- 35 Martin GJ, Magid NM, Eckberg DL *et al.* Heart rate variability and sudden cardiac death during ambulatory monitoring. *Clin Res* 1987; **35**: 302A. (Abstract)
- 36 Morgan HE, Baker KM. Cardiac hypertrophy: mechanical, neural and endocrine dependence. *Circulation* 1991; **83**: 13–25.
- 37 Kelm M, Schäfer S, Mingers S *et al.* Left ventricular mass is linked to cardiac noradrenaline in normotensive and hypertensive patients. *J Hypertens* 1996; **14**: 1357–64.
- 38 O'Brien IA, O'Hare P, Corrall RJM. Heart rate variability in healthy subjects: effect of age and the derivation of normal ranges for tests of autonomic function. *Br Heart J* 1986; **55**: 348–54.
- 39 Espi F, Ewing DJ, Clarke BF. Testing for heart rate variation in diabetes: single or repeated deep breaths? *Acta Diabetol Lat* 1982; **19**: 177–81.

Received 18 March 1998; accepted 13 August 1998.

Correspondence: Dr Ole May, Skovallen 10, 5250 Odense SV, Denmark (fax + 4566118796; e-mail olemay@dadlnet.dk).