New method for assessing cardiac parasympathetic activity using 24 hour electrocardiograms

DAVID J EWING, JAMES M M NEILSON, PAUL TRAVIS

From the University Departments of Medicine and of Medical Physics and Medical Engineering, Royal Infirmary, Edinburgh

SUMMARY Cardiac parasympathetic activity was assessed using 24 hour electrocardiographic recordings by measuring the incidence of larger changes in successive RR intervals, which in normal subjects occur frequently but irregularly. In 25 normal subjects the mean number of times per hour in which the change in successive RR interval was greater than 50 ms was 150–250 during waking and 350–450 during sleeping. By contrast, 30 diabetics with medically denervated hearts (12 with cardiovascular reflex evidence of parasympathetic damage and 18 with additional sympathetic damage) and six cardiac transplant patients with surgically denervated hearts had extremely low counts. Additionally, of 20 diabetics with normal cardiovascular reflexes, about half had abnormally low counts, suggesting that this method is better than currently available reflex tests in detecting early cardiac parasympathetic damage. This technique provides a valid and sensitive way of monitoring cardiac parasympathetic activity over prolonged periods.

Heart rate regulation is mainly dependent on autonomic innervation. Although efferent cardiac vagus nerve activity cannot be directly measured in conscious humans, there is good evidence from animal studies that the degree of respiratory sinus arrhythmia is directly related to efferent vagal traffic.[1-3] Cutting the vagus nerve in animals abolishes variation in heart rate.[4] In man abrupt changes in heart rate, such as those during muscular exercise,[5 6] standing up,[7] and lying down,[8] are mediated by the vagus nerve, since blocking by atropine abolishes them, whereas they are unaffected by propranolol. Clinically, therefore, heart rate variation, usually in response to specific stimuli, is used to assess cardiac vagal (or parasympathetic) integrity. At present, several simple tests are available, such as the heart rate responses to the Valsalva manoeuvre, deep breathing, and standing.[9] Individual results are, however, sometimes difficult to interpret as measurements are made only over very short time spans and a single test may give an atypical result owing to natural variability.

We report a new method of measuring cardiac parasympathetic activity using heart rate variability in

response to natural background stimuli. The technique requires no subject collaboration and may be used to monitor variations throughout the day and night. It arose from our observation that 24 hour recordings of RR interval in patients with autonomic damage were noticeably smoother than those in normal subjects, which on many occasions showed abrupt changes in RR interval with differences reaching several hundred milliseconds. The normal recording (Fig. 1a) has a much spikier appearance since superimposed on the cyclical changes of sinus arrhythmia are larger and irregular "steps." By contrast, the diabetic tracing (Fig. 1b) shows a smooth sequence of much smaller interval changes. If only steps exceeding a 50 ms increase or decrease in RR interval are counted 150 to 400 increasing steps per hour and a similar number of decreasing steps are normally seen.

Subjects and methods

STUDY POPULATION
Twenty five normal subjects aged 20–65 (mean 37) years were studied once. Eleven underwent repeat 24 hour electrocardiographic monitoring some weeks later. Six other subjects had their recordings repeated while taking their sixth daily dose of an oral beta adrenergic blocking drug, propranolol (320 mg) (Inderal-LA, ICI Pharmaceuticals). Twelve diabetic

Correspondence to Dr D J Ewing, Department of Medicine, Royal Infirmary, Edinburgh, EH3 9YW. (Reprints will not be available.)
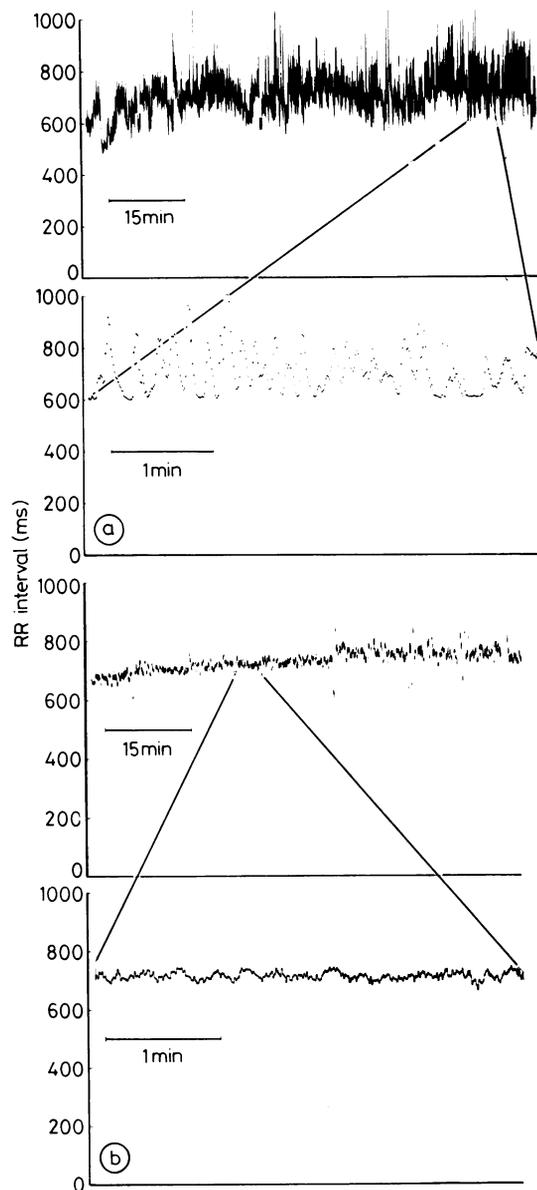
Fig. 1   RR interval recordings in (a) a young normal subject showing a spiky appearance (upper trace) made up of both smaller and larger RR interval steps (lower trace) and (b) an age matched diabetic patient with autonomic damage showing a much smoother appearance (upper trace) made up of smaller RR interval steps only (lower trace).

patients aged 25–62 (mean 47) years with abnormal cardiovascular reflexes suggestive of cardiac parasym- for comparison with the normal subjects. It was pre-

pathetic damage (diabetic group 1) were monitored sumed that these subjects represented a group with medically denervated hearts, in which the parasym- pathetic nerve supply had been eliminated. The 24 hour recordings from six patients aged 23–46 (mean 34) years after cardiac transplantation were also studied, thus enabling comparison with a group with surgically denervated hearts. Two other groups of diabetic patients were also studied: 20 aged 25–59 (mean 43) years with normal cardiovascular reflexes (diabetic group 2) and 18 aged 28–64 (mean 42) years with abnormal cardiovascular reflexes suggestive of both parasympathetic and additional sympathetic damage (diabetic group 3).

The diabetic patients were grouped according to their responses to a standardised battery of five car- diovascular autonomic function tests[9-11]: heart rate responses to the Valsalva manoeuvre, deep breathing, and standing up, testing cardiac parasympathetic integrity, and blood pressure responses to standing up and sustained handgrip, which are abnormal only when there is more extensive sympathetic damage.

ELECTROCARDIOGRAPHIC MONITORING
For each subject a miniature tape recorder (Medilog 1, Oxford Medical Systems Limited) was used to record a single lead electrocardiogram with a time-frequency reference signal simultaneously recorded on an adjacent tape track. Recording continued for 24 hours while the subject undertook his normal work and leisure activities. Times of going to bed and rising were noted by the subjects. There were no significant differences between the different groups in the number of hours awake or asleep.

The tapes were later replayed through a Pathfinder high speed arrhythmia analyser (Reynolds Medical Limited) at exactly 60 times the original recording speed to detect extrasystoles and generate outputs representing heart rate and beat by beat RR interval. The time reference track was used to adjust the speed up ratio to within 0·5%. To facilitate the subsequent RR interval analysis, only recordings showing normal sinus rhythm and less than five extrasystoles per hour were analysed further.

The recordings were again replayed, and the analogue RR interval waveform electronically dif-ferentiated to generate pulses proportional to the change of RR interval at each complex. Separate pulses were generated for positive and negative increments in RR interval, and each of the two pulse trains passed to a comparator which generated an out-put pulse whenever the pulse amplitudes indicated a change of RR interval greater than the preset threshold value of 50 ms. These output pulses were counted electronically to give the total number of positive and negative suprathreshold RR interval
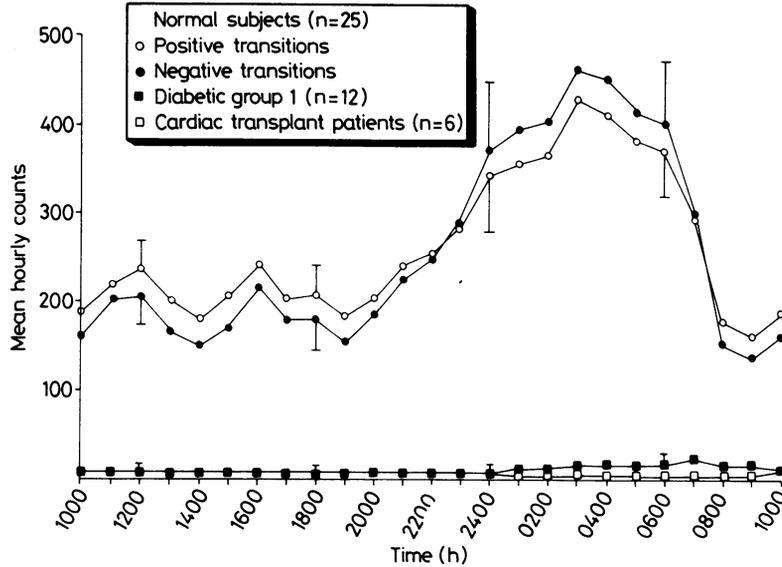
Fig. 2 *Group arithmetic mean hourly RR interval step counts in normal subjects, diabetic patients with medically denervated hearts (group 1), and cardiac transplant patients with surgically denervated hearts. Bars represent SEM.*

steps per hour. Measurements of the apparent RR interval variation of constant frequency electronically generated pulses, which had been recorded and replayed in the same way as the electrocardiograms, showed a standard deviation of successive RR interval changes of 10 ms. Fifty measurements of the 50 ms step counts during a given one hour segment of the recording were obtained for each of six subjects (coefficient of variation 4%).

Selected recordings were further analysed using a different threshold value to trigger counts: at one sixteenth (6·25%) of the immediately preceding RR interval. Using this method a smaller threshold applied when the RR interval was shorter and a larger threshold when it was longer. The actual value of 6·25% is equivalent to a 50 ms step when the RR interval is 800 ms, to a 62·5 ms step at 1000 ms, and to a 37·5 ms step at 600 ms.

STATISTICAL ANALYSIS

To simplify the analysis the mean step rate in counts per hour over the waking and sleeping periods was calculated for each subject. The distribution of step rates among the subjects studied was notably skew, and for analytical convenience a logarithmic transformation was applied. Standard parametric statistical techniques were then used on the transformed rates. Group mean values for total, mean waking, and mean sleeping step counts are expressed as geometric means.
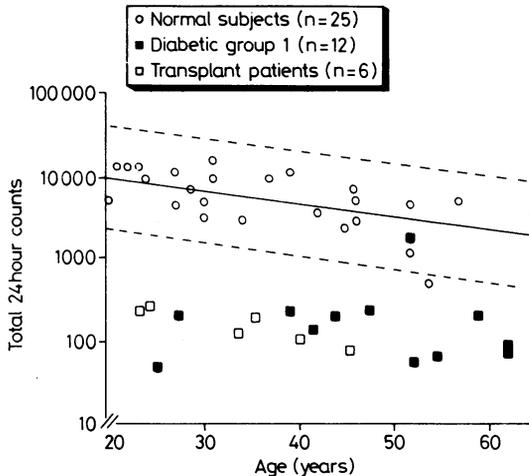

Fig. 3 *Relation between total RR interval step counts over 24 hours and age in 25 normal subjects, 12 diabetic patients with medically denervated hearts (group 1), and six cardiac transplant patients with surgically denervated hearts. The dotted lines represent the 95% tolerance limits for the normal subjects.*
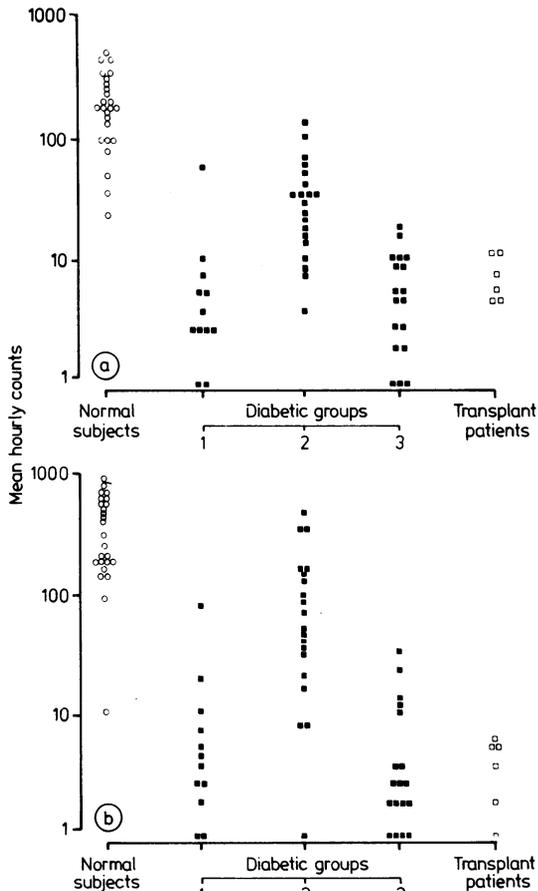
Fig. 4 *Individual mean hourly RR interval step counts (a) awake and (b) asleep in 25 normal subjects, 12 diabetics with cardiac parasympathetic damage (group 1), 20 diabetics with normal cardiovascular reflexes (group 2), 18 diabetics with cardiac parasympathetic and additional sympathetic damage (group 3), and six cardiac transplant patients.*

Results

NORMAL SUBJECTS

Figure 2 shows the group mean hourly counts for both positive and negative transitions in the normal subjects. The daytime counts averaged approximately 200 per hour, whereas the counts at night were higher and averaged between 350 and 450 counts per hour. When the total counts for the whole 24 hours period and the mean hourly counts for the hours awake and hours asleep were considered there were no significant differences between positive and negative steps, nor was there any significant interaction between the waking and sleeping periods and the positive and negative steps. In view of this, only the positive steps (referred to hereafter as counts) have been included in the subsequent analysis.

Figure 3 shows the relation between the total 24 hour counts and age. There was a significant inverse relation with age ($r = -0.59$, $p < 0.001$). A similar relation was found with the mean waking ($r = -0.64$) and mean sleeping ($r = -0.55$) counts. Figure 4 shows the individual values of the mean hourly counts awake and asleep plotted on a logarithmic scale. The sleeping values were significantly higher than the waking values in normal subjects ($t = 4.8$, $p < 0.001$) with only three of the 25 subjects having higher mean counts during the day.

Eleven normal subjects had their 24 hour recordings repeated. There were no statistically significant differences between the mean levels of any of the counts on the first and second days of recording in this group. In contrast, a group of six other normal subjects showed statistically significant differences between observations made before and during beta blockade both for total counts and for waking counts. The mean total counts increased from 5248 to 8995 during beta blockade ($t = 3.2$, $p < 0.05$) and mean hourly waking counts increased from 152 to 324 ($t = 4.8$, $p < 0.01$). Although sleeping counts showed some increase, the difference was not statistically significant (from 334 to 472, $t = 2.1$, NS).

DIABETIC PATIENTS

The group mean hourly counts, the total 24 hour counts, and the individual waking and sleeping values for diabetics with cardiovascular reflex evidence of parasympathetic damage (diabetic group 1) were well outside the normal range in all but one subject (Figs. 2–4) and outside the 95% tolerance limits when age was taken into consideration as well (Fig. 3) Unlike the normal subjects there were no significant correlations between age and total, waking, or sleeping counts. Figure 4 also shows the mean hourly counts of the two other diabetic groups. Those with additional sympathetic damage (group 3) were indistinguishable from those with parasympathetic damage (group 1). In those with normal cardiovascular reflexes (group 2), however, about half had results at the lower end of the normal range but the remainder had extremely low counts that were clearly abnormal.

TRANSPLANT PATIENTS

The six transplant patients also had very low counts, which could not be distinguished from those of the diabetic patients with parasympathetic damage (group 1) (Figs. 2–4).

MEAN HEART RATE AND COUNTS

The Table shows the group mean waking and sleeping heart rates in each of the five groups. There was a

Table   *Group mean heart rates and hourly counts awake and asleep in the normal subjects, diabetic patients, and transplant patients. Figures are mean (SD and range)*

Group	Heart rate (beats/min)		Hourly counts (geometric mean)	
	Awake	Asleep	Awake	Asleep
Normal subjects (n=25)	83(7)	59(7)	170	275
	(72–96)	(50–72)	(25–486)	(11–969)
Diabetic patients*				
Group 1 (n=12)	95(11)	76(8)	4	5
	(71–111)	(63–90)	(1–61)	(0–87)
Group 2 (n=20)	92(8)	68(8)	28	57
	(77–105)	(53–86)	(4–142)	(1–499)
Group 3 (n=18)	96(12)	81(13)	5	3
	(79–125)	(60–107)	(1–17)	(0–36)
Transplant patients (n=6)	101(12)	91(15)	7	3
	(87–114)	(75–113)	(5–12)	(2–7)

*Group 1, those with cardiovascular reflex of parasympathetic damage; group 2, those with normal cardiovascular reflexes; group 3, those with cardiac parasympathetic and additional sympathetic damage.

wide range of individual heart rates in the normal and diabetic groups. The transplant patients had slightly higher heart rates. Despite the considerable overlap in heart rates between those with and without cardiac parasympathetic damage, there was a wide separation in the number of counts obtained with the normal subjects having high counts and the diabetics with cardiac parasympathetic damage (groups 1 and 3) extremely low counts (Table). The normal subjects were also arbitrarily divided into those under 40 (n=15) and over 40 years (n=10). Although the mean hourly counts were much lower in the older group (under 40: awake 243, asleep 443; over 40: awake 97 (p<0.01), asleep 137 (p<0.01)) the mean heart rate values waking and sleeping were very similar (under 40: awake 84, asleep 59; over 40: awake 82 (NS), asleep 61 (NS)).

COMPARISON OF STEP COUNTS
The results of the 6.25% step counts were compared with the 50 ms step counts in 10 normal subjects and three diabetics with cardiac parasympathetic damage. In the normal subjects the use of the 6.25% threshold had the effect of increasing the mean hourly waking counts from 168 (50 ms) to 220 (6.25%). It also reduced the mean hourly sleeping counts from 361 (50 ms) to 244 (6.25%) The total 24 hour counts were, however, very similar using both methods: 5546 (50 ms) and 5520 (6.25%). In the three diabetics with very low counts there was hardly any change in counts with a mean total increase from 50 to 81 counts over the whole 24 hours and an increase in the waking counts from two to four per hour.

Discussion

Sudden changes in heart rate occur irregularly, but frequently, throughout the day and night in normal subjects. They may be detected in addition to other slower variations in heart rate, such as respiratory sinus arrhythmia[12 13] and the very small beat by beat changes in RR interval that are occurring continuously. Methods of quantifying heart rate variation have previously relied on such measures as standard deviation, mean of successive RR interval differences, or differences between maximum and minimum heart rates during a respiratory cycle,[14] all of which mainly reflect these smaller changes. Instead, therefore, of measuring these small changes, this new approach measures the number of changes in RR interval over a particular magnitude that are occurring per unit time. We chose a threshold value of 50 ms difference from the preceding RR interval, as this level gave a very clear separation between normal subjects and those with cardiac parasympathetic damage. Had we chosen a value of 45 ms or 55 ms, our results would have been very similar. In view of the episodic nature of these larger steps, we selected a counting period of one hour to smooth out the distribution of counts and to allow sufficient numbers to accumulate. The technique is, of course, inappropriate in patients who have either an arrhythmia or a large number of extrasystoles. We arbitrarily took a rate of less than five extrasystoles per hour as acceptable.

Our results also show what happens to the counts when the threshold value was changed to a percentage difference of the immediately preceding RR interval instead of fixing it at 50 ms. At a faster heart rate a smaller interval difference was needed to trigger counts and at a slower heart rate a larger difference. Accordingly, therefore, the waking counts—where the heart rate was faster—were higher and the sleeping counts lower, but the total 24 hour counts largely unchanged. In those with cardiac parasympathetic damage, changing the threshold to the percentage method made no difference to the counts and thus did not alter the interpretation of the results. Whether a

fixed threshold or a percentage threshold is chosen is, of course, arbitrary. Previous animal studies have clearly shown the proportional relation between variation in RR interval and efferent vagal activity.[1-3] There is, however, no evidence available linking the prevailing heart rate, the size of the step change in RR interval between complexes, and the number of nerve impulses passing down the efferent vagus nerve. Either threshold method is acceptable given the present evidence since both distinguish clearly between normal and abnormal results. For this study we have chosen the 50 ms fixed threshold as it is easier and simpler to measure than a percentage threshold.

In addition to the evidence from previous animal and human studies, there are further good reasons for assuming that these RR interval steps are mediated by the vagus nerve. We found that the number of steps decreased with age, possibly corresponding to a decrease in vagal tone in older subjects. The number of counts we recorded increased at night, and this is probably because during the day both sympathetic and parasympathetic influences change the heart rate, whereas at night—except during periods of rapid eye movement—activity of the vagus nerve is dominant and there is little sympathetic outflow.[15] Other evidence for our recordings being an actual measure of cardiac parasympathetic activity comes from our beta adrenergic blocking studies in normal subjects, in whom the counts were not diminished by beta blockade. Assuming that the beta blockade was fairly complete, the remaining neural control of heart rate during the 24 hours must depend solely on the fast acting vagal system modifying the underlying intrinsic cardiac rate. During the day, with beta blockade, heart rate changes required for different activities will be produced only via the vagus nerve, resulting in jerky control and hence increased daytime counts. At night, by contrast, because of the low sympathetic activity beta blockade alters the counts very little.

The best evidence that we are measuring cardiac parasympathetic activity directly comes from the results of the diabetic patients with cardiovascular reflex evidence of vagal damage, who can be considered to have medically denervated hearts, and the transplanted patients, who undoubtedly have surgical cardiac denervation. In both of these groups the counts were almost zero, and there were no differences in the number of counts between the waking and sleeping periods.

The variations in counts that normally occur throughout the day and night are, therefore, an index of spontaneous alterations of efferent vagal activity. These may partly be a response to external stimuli such as muscular exercise, postural changes, or respiration and partly a response to unknown factors arising within the central nervous system. Our subjects were fully ambulant and moving around during the day. At night they were sleeping, and although spontaneous movements, of course, often occur during sleep, these would not appear to be enough to explain the considerable increase in counts in normal subjects. We have not attempted to analyse the interplay of any of these various factors in any way.

The relation between the underlying heart rate and the heart rate variation is a complex one. The two are clearly associated as both are affected by changes in cardiac vagal activity. Most previous studies have considered that heart rate is a relatively good, if rather crude, index of cardiac parasympathetic function, with low heart rates representing increased activity and higher heart rates representing reduced activity. We consider that by measuring steps in RR interval as described we have a better measure of cardiac parasympathetic activity, in which the separation between normal and abnormal is much more clear cut. It might be suggested that this measure of RR interval variation that we describe is only a rather sophisticated measure of heart rate. Our results do not support this since, for example, there is an overlap in heart rates but a wide separation of counts between the normal subjects and the diabetics with parasympathetic damage. Similarly, the older normal subjects, although showing much lower counts, still had heart rate values that were similar to their younger counterparts.

Thus this method of measuring counts over prolonged periods seems to provide a good measure of cardiac parasympathetic activity. There are several possible applications of this technique in various clinical fields. The results in the diabetic patients show that there were several with abnormally low counts despite normal conventional cardiovascular reflex tests. This suggests that this method will be more sensitive than currently available reflex tests in detecting early cardiac parasympathetic damage. Other work currently in progress suggests that it will also have considerable uses in following the time course of events and recording continuously the varying levels of cardiac parasympathetic activity throughout the day and night, for example, after myocardial infarction.

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