Heart Rate–Dependent Hysteresis of T-Wave Alternans in Primary Prevention ICD Patients

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Background: T-wave alternans (TWA) is usually performed at accelerated heart rates (HR) during exercise, while recovery TWA is typically not analyzed. Consequently, it is still unknown if TWA shows a HR-dependent hysteresis or not. Thus, the aim of the present study was to investigate TWA dependency on HR during both the exercise and recovery phases of an ergometer test, and to evaluate if recovery TWA may contribute to identify subjects at increased risk of arrhythmic events.

Methods: Our HR adaptive match filter was used to identify TWA from electrocardiographic recordings acquired during a bicycle ergometer test in 266 patients with implanted cardio-defibrillator. During the 4-year follow-up, 76 patients developed tachycardia or ventricular fibrillation (ICD_Cases) and 190 did not (ICD_Controls).

Results: TWA was statistically lower during exercise than recovery for HRs between 75 and 110 bpm (16–21 μ V vs 20–27 μ V; $P < 0.05$), and reverse for HRs between 120 and 130 bpm (41–51 μ V vs 28 μ V; $P < 0.05$). ICD_Cases and ICD_Controls showed significantly different TWA at 80 bpm (20 μ V vs 15 μ V; $P < 0.05$) and 140 bpm (15 μ V vs 22 μ V; $P < 0.05$) during exercise, and at 90 bpm (38 μ V vs 21 μ V; $P < 0.05$) and 95 bpm (33–24 μ V vs 28 μ V; $P < 0.05$) during recovery.

Conclusions: TWA shows a HR-dependent hysteresis and there is a different behavior of TWA in ICD_Cases and ICD_Controls groups. Consequently, beside exercise TWA also recovery TWA may contribute to identify subjects at increased risk of arrhythmic events.

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Microvolt T-wave alternans (TWA) consists in a subtle every-other-beat fluctuation of the electrocardiographic (ECG) T-wave amplitude at stable heart rate (HR) during sinus rhythm, and is considered a promising noninvasive index to predict the occurrence of malignant ventricular arrhythmias and sudden cardiac death.^{1–8} It has been observed that TWA tends to increase its amplitude with increasing HR^{8–11} so that, even though it has also been observed in resting conditions,^{5,12–15} TWA analysis is often performed at accelerated

HRs reached through exercise.^{1,4,5,7–9,16,17} Several studies have shown that exercise-induced TWA is capable of stratifying risk for cardiovascular death and lethal arrhythmias.^{1,4,5,16–18} However, other studies have not confirmed the usefulness of TWA in risk stratification, but rather conclude that the TWA-related benefits seem to vary with studied population.^{19–21} In a recent document providing a consensus guideline for TWA, authors conclude that even though it is reasonable to consider TWA evaluation whenever there is suspicion of

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vulnerability to lethal cardiac arrhythmias, there is as yet no definite evidence from interventional trials that it can guide therapy.²² Thus, further investigations are needed before exercise TWA incremental prognostic role can be properly defined.

When evaluated during an exercise testing, TWA is typically measured during the HR-increasing phase of the test or at fast HRs (100–125 bpm) in correspondence of which it significantly increases its amplitude.^{1, 4, 5, 7–9, 16, 17} Still, any exercise test also includes a recovery phase, characterized by a decreasing HR, during which the patient returns to his/her initial conditions. Recovery TWA has been rarely reported.⁵ Consequently, it is not clear if it there exists a HR-TWA one-to-one correspondence, or if TWA shows a HR-dependent hysteresis (that is, the TWA value at a certain HR depends on how such HR has been reached). Thus, the aim of the present study was to investigate the dependency of TWA from HR during both the HR-increasing exercise and the HR-decreasing recovery phases of a test, and to evaluate if recovery TWA can provide further information to improve identification of patients at increased risk of major arrhythmic events.

METHODS

Study Populations and Clinical Data

The present study was performed on the Leiden University Medical Center Database, which consists in the collection of routine clinical data and exercise ECG from 266 patients with an implanted cardiac defibrillator (ICD) for a depressed left ventricular ejection fraction (LVEF). More specifically, LVEF had to be less than 35% for a patient to receive the implantation. According to "Guideline for Good Clinical Practice" (European Medicines Agency, CPMP/ICH/135/95) and the data privacy law in the Netherlands, no informed consent from patients was needed for being enrolled in the present retrospective and observational study, since patients remain anonymous, only standard clinical data are used, and no experimental interventions have taken place.

Patients follow-up lasted 4 years starting from the ICD implantation date. During the follow-up patients were receiving standard care, which

included periodic visits to the outpatient clinic for ergometry assessment through bicycle tests, during which 12 standard ECG recordings were obtained using a CASE 8000 stress test recorder (GE Healthcare, Freiburg, Germany; sampling frequency: 500 Hz; resolution: 4.88 μ V/LSB) and 3M Red Dot ECG Electrode Soft Cloth 2271 electrodes, specifically meant for stress testing. The skin was first cleaned with alcohol and then abraded to reduce electrode resistance, which was measured by the electrocardiograph and considered acceptable if <5 kOhm. Electrodes were applied according to the Mason-Likar configuration [<http://www.ncbi.nlm.nih.gov/pubmed/5902099>].

Each ergometer test included two phases: exercise and recovery. The exercise phase consisted in an approximately 10-minute bicycle test during which the workload was incremented from zero to the patient's maximal exercise capacity by applying load increments of about 10% of the expected maximal exercise capacity every minute. The recovery phase lasted approximately 2–5 minutes; initially the workload was gradually but quickly decremented to 20 W within 1 minute; then the patient was asked to cycle for another 1–2 minutes, and eventually he/she had to wait on the bike until his/her HR was close to that at the beginning of the test. Thus, each ECG was characterized by an increasing HR during the exercise phase of the test, and by a decreasing HR during the recovery phase. The maximum reached HR (MaxHR) identified the exact instant when exercise ended and recovery started.

According to the data-collection protocol, during the follow-up patients could perform more than one ergometer test. At the end of the follow-up, patients were classified as either ICD-Cases (76 patients) if, during the follow-up, they had developed ventricular tachycardia or ventricular fibrillation (VT/VF; treated with antitachycardia pacing and/or shock therapy), or ICD-Controls (190 patients) if no device therapy had emerged. ICD-Cases ergometer tests were excluded when a major cardiac event (infarction, VT ablation, coronary artery bypass graft) occurred between the test and the moment of VT/VF, since ablation potentially modified their arrhythmogenic substrate with respect to the state in which it was when the ICD was implanted. If more than one ergometer test remained available, the one closest in time to the VT/VF episode was selected so that the electrophysiological properties of the arrhythmogenic substrate during the stress

test would be as close as possible to those just preceding the actual arrhythmia. In ICD.Controls with more than one suitable exercise test, the earliest was selected. Eventually, only one ECG tracing per patient was made available for the database.

TWA Identification

TWA was evaluated in each precordial lead independently. Only the six precordial leads were used because previously found optimal for this ICD population.^{5,23} The analysis was performed in a completely automatic way using the B.M.E.D. (Bio-Medical Engineering Development, SRL, Ancona, Italy, www.bmed-bioengineering.com) software implementation of our HR adaptive match filter (AMF) based technique²⁴⁻²⁷ by individuals who were blinded to outcomes.

Each ECG lead was preprocessed for noise removal (0.5–35 Hz bandpass filter) and baseline subtraction (by subtraction of a third-order spline interpolation).²⁴ ECG windows including 64 consecutive beats were recursively extracted every 2 seconds from the entire recording and processed for artifacts and ectopic beats replacement.^{24,25} Only ECG windows characterized by stable HR (NN standard deviation $\leq 10\%$ mean NN) and a low number (≤ 4) of replaced beats were considered suitable for TWA analysis and classified according to test phase (exercise vs recovery) and HR. Seventeen different HR levels were considered, respectively, from 70 to 150 bpm, each covering a 5-bpm range (i.e., a HR belonged to the HR_i level if $HR_i - 2.5 \text{ bpm} \leq HR < HR_i + 2.5 \text{ bpm}$, with $HR_i = 70, 75 \dots 150 \text{ bpm}$). Eventually, TWA was identified in every suitable ECG window by submitting it to the AMF (see below).²⁴⁻²⁷ If few suitable ECG windows of the same lead were available for a HR level, their TWA measurements were averaged. Eventually, a TWA characterization for each HR was provided in terms of maximum TWA over the six precordial leads.

HR Adaptive Match Filter

In the ideal condition of a perfectly constant HR TWA is characterized by a single frequency which, by definition, is equal to half HR. Real ECG signals, however, are characterized by a certain amount of HR variability and, in the presence of sinus rhythm, TWA can still be defined but its frequency

content will be a small band around twice mean HR rather than a single frequency. Consequently, our AMF-based method automatically identifies TWA by filtering the ECG signal with a narrow-band passing filter.²⁴⁻²⁷ More specifically, when fed with an ECG, the AMF first computes the mean HR and thus the central TWA frequency ($f_{TWA} = 1/(\text{mean HR})$), and then identifies the passing band as $f_{TWA} \pm df_{TWA}$, with $df_{TWA} = 0.06 \text{ Hz}$ (value empirically found). The AMF implementation consists of a sixth-order bidirectional Butterworth bandpass filter characterized by a 0.12 Hz wide passing band realized as cascade of a lowpass filter (LPF; cutoff frequency $f_{LPF} = f_{TWA} + df_{TWA}$) and a highpass filter (HPF; cutoff frequency $f_{HPF} = f_{TWA} - df_{TWA}$). Its transfer-function squared module is the following:

$$|H_{AMF}(f)|^2 = |H_{LPF}(f)|^2 \cdot |H_{HPF}(f)|^2$$

$$= \frac{1}{1 + \left(\frac{f}{f_{LPF}}\right)^6} \cdot \frac{\left(\frac{f}{f_{HPF}}\right)^6}{1 + \left(\frac{f}{f_{HPF}}\right)^6}. \quad (1)$$

The AMF filters out every ECG component but TWA, and provides an amplitude-modulated pseudo-sinusoidal signal, termed TWA-signal, as output. If really pertaining to TWA (and not, for example, to QRS oscillations at f_{TWA}), the TWA-signal maxima and minima fall inside the repolarization segment (ST segment and T-wave). If this is the case, the TWA-signal amplitude quantifies TWA (μV), otherwise TWA is set to zero.

Statistics

Comparison between clinical and/or TWA parameters relative to ICD groups were performed using parametric and nonparametric tests for normal and not-normal distributions, respectively. Distribution normality was tested using the Lilliefors test. Normally distributed parameters (reported in terms of mean $\pm$ standard deviation) were compared using the *t*-test for equal means, whereas not-normally distributed parameters (reported in terms of median [25th; 75th percentiles]) were compared using the Wilcoxon rank-sum test for equal medians. Differences in the binary parameters (reported in terms of number of occurrences) distributions between the two groups were evaluated using the chi-square test. The

statistical significance level was set at 5% in all cases.

RESULTS

The general clinical profile at the time of the exercise test of the ICD population is reported in Table 1, together with the clinical information relative to the two subgroups. ICD_Cases and ICD_Controls were clinically comparable, with the only exception of the LVEF that was significantly lower in ICD_Cases than ICD_Controls ($31 \pm 12\%$ vs $39 \pm 12\%$, $P < 10^{-5}$; Table 1). On average, the exercise phase of the bicycle ergometric test lasted 8.8 minutes, while the recovery lasted 4.3 minutes (Table 1). The mean HR at the beginning of the test was 80 bpm, while at the end of it was 86 bpm, so that HR was usually not completely recovered ($P < 0.05$). Eventually, MaxHR was comparable in the two ICD groups (ICD_Cases: 126 ± 18 bpm vs ICD_Controls: 127 ± 17 ; $P > 0.05$).

When considering the entire ICD population, the mean TWA amplitude during exercise was comparable to that during recovery ($30 \mu\text{V}$ vs $32 \mu\text{V}$; Table 1). The way TWA varied with HR, however, was different in the two phases of the test. Figure 1 displays plots of HR during the bicycle ergometer test and measured TWA as functions of time for a patient whose MaxHR was 125 bpm (Patient A, Fig. 1a,b) and for a patient whose MaxHR was 140 bpm (Patient B, Fig. 1c,d). In both cases, TWA remained quite low and practically constant for HRs below 105–110 bpm, reached its maximum value at 125 bpm and then tended to return to its initial value. By identifying the MaxHR as the end of the exercise phase, TWA peaks occurred in correspondence of the exercise-recovery transition in Patient A, and in the middle of exercise in Patient B. This figure, together with Figure 2a that displays a plot of mean TWA as function of HR during the ergometer test for the entire ICD population, indicate a different behavior of TWA with test phase. During the HR-increasing exercise phase, TWA remained practically constant at baseline levels for HRs lower than 110 bpm; then it significantly increased with HR for HRs between 110 and 125 bpm; and eventually it decreased till its baseline levels for HRs greater than 125 bpm. During the HR-decreasing recovery phase, TWA slightly increased for HRs between 145 and 130 bpm, and then

it gradually returned to its baseline levels. As a consequence, exercise TWA was significantly lower than recovery TWA for HRs between 75 and 110 bpm ($P < 0.05$ for $75 \text{ bpm} \leq \text{HR} \leq 85 \text{ bpm}$; $P < 10^{-3}$ for $\text{HR} = 90$ and $105 \text{ bpm} \leq \text{HR} \leq 110 \text{ bpm}$; and $P < 10^{-6}$ for $95 \text{ bpm} \leq \text{HR} \leq 100 \text{ bpm}$; Table 2; Fig. 2a), while the opposite occurred for HRs between 120 and 130 bpm ($P < 10^{-6}$ for $120 \text{ bpm} \leq \text{HR} \leq 125 \text{ bpm}$; $P < 10^{-3}$ for $\text{HR} = 130 \text{ bpm}$; Table 2; Fig. 2a).

When analyzing the two ICD groups separately, they showed some significant differences with regard to TWA dependency on HR. The exercise-recovery TWA trends described above for the entire ICD population (Fig. 2a) was completely confirmed for ICD_Controls (Fig. 2c), which was the most numerous group (190 patients out of 266), but not for ICD_Cases (Fig. 2b). Specifically, in the latter group, the exercise TWA increment for HRs between 115 and 135 bpm was less marked so that no significant differences between exercise and recovery TWA were observed in that HR range (Table 2; Fig. 2b). Moreover, in ICD_Cases TWA during recovery did not gradually decreased implying recovery TWA to be significantly higher than exercise TWA only for HR between 90 and 110 bpm ($P < 10^{-3}$ for $90 \text{ bpm} \leq \text{HR} \leq 95 \text{ bpm}$; and $P < 0.05$ for $100 \text{ bpm} \leq \text{HR} \leq 110 \text{ bpm}$; Table 2; Fig. 2b). Eventually, only in ICD_Cases mean recovery TWA was significantly higher than mean exercise TWA ($37 \mu\text{V}$ vs $31 \mu\text{V}$, $P < 0.05$; Table 1).

Not all HRs or test phases were equally useful to discriminate the two ICD groups in terms of TWA (Table 2). During exercise TWA was significantly higher in ICD_Cases than ICD_Controls at 80 bpm ($20 \mu\text{V}$ vs $15 \mu\text{V}$, $P < 0.05$; Table 2), and reverse at 140 bpm ($15 \mu\text{V}$ vs $22 \mu\text{V}$, $P < 0.05$; Table 2). Instead, during recovery significant TWA differences between ICD_Cases and ICD_Controls were observed at 90 bpm ($38 \mu\text{V}$ vs $21 \mu\text{V}$, $P < 0.05$; Table 2) and 95 bpm ($33 \mu\text{V}$ vs $24 \mu\text{V}$, $P < 0.05$; Table 2).

DISCUSSION

This retrospective, observational study on standard clinical data investigated the dependency of TWA from HR during both HR-increasing exercise and HR-decreasing recovery phases of an ergometer test, and evaluated if recovery TWA, usually not analyzed, can provide further

Table 1. General Clinical Information Relative to the ICD Population and Its Two Subgroups, the ICD_Cases and the ICD_Controls

	ICD (266)	ICD_Cases (76)	ICD_Controls (190)	P
General:				
Gender (male)	213 (80%)	61 (80%)	152 (80%)	NS
Age (years)	60 ± 12	59 ± 11	61 ± 12	NS
BMI (kg/m ²)	27 ± 4	27 ± 4	26 ± 4	NS
LVEF (%)	37 ± 13	31 ± 12	39 ± 12	<10 ⁻⁵
Therapies:				
CRT_D	130 (49%)	40 (53%)	90 (47%)	NS
NYHA functional class:				
I;II	220 (83%)	60 (79%)	160 (84%)	NS
III;IV	46 (17%)	16 (21%)	30 (16%)	NS
Medications:				
Beta-blocker	231 (87%)	68 (89%)	163 (86%)	NS
Amiodarone	31 (12%)	15 (20%)	16 (8%)	<0.05
Calcium antagonists	16 (6%)	2 (3%)	14 (7%)	<0.05
Flecainide	1 (0%)	1 (1%)	0 (0%)	NS
Digoxin	22 (8%)	11 (14%)	11 (6%)	<0.05
ACE inhibitor / AT antagonist	242 (91%)	69 (91%)	173 (91%)	NS
Diuretics for CHF	194 (73%)	61 (80%)	133 (70%)	NS
Statins	199 (75%)	58 (76%)	141 (74%)	NS
Ergometry:				
HR at rest (bpm)	71 ± 11	72 ± 10	71 ± 11	NS
Exercise starting HR (bpm)	80 ± 11	81 ± 11	81 ± 11	NS
Maximum HR (bpm)	127 ± 18	126 ± 18	127 ± 17	NS
Recovered HR (bpm)	86 ± 12	85 ± 14	86 ± 12	NS
Exercise duration (min)	8.8 ± 3.7	8.3 ± 3.3	9.0 ± 3.9	NS
Recovery duration (min)	4.3 ± 1.9	4.2 ± 1.5	4.4 ± 2.1	NS
T-wave alternans:				
Mean exercise TWA (μV)	30 ± 19	31 ± 25	29 ± 17	NS
Mean recovery TWA (μV)	32 ± 20	37 ± 24	31 ± 18	NS

Values were obtained at the time of the exercise test and are reported in terms of mean ± standard deviation or number of occurrences.

*P < 0.05 when comparing clinical parameter in ICD_Cases versus ICD_Control.

ACE = angiotensin converting enzyme; AT = angiotensin; BMI = body mass index; CHF = congestive heart failure; CRT_D = cardiac resynchronization therapy with defibrillator; HR = heart rate; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association; NS = not statistically significant; TWA = T-wave alternans.

information to improve identification of patients at increased risk of major arrhythmic events. To this aim the Leiden University Medical Center database of exercise ECGs in heart failure patients with an ICD was used. The database includes ECG tracings from 266 ICD patients of which 76 developed VT/VF during the 4-year follow-up (ICD_Cases) and were treated with antitachycardia pacing and/or shock therapy, while the remaining 190 did not (ICD_Controls). It is well-known that antitachycardia pacing is not a good surrogate for really malignant ventricular arrhythmias episodes, and ICD-shock is not a good surrogate for sudden cardiac death. This problem plays an important role in the recent discussion about the controversial results of TWA in studies with ICD patients versus pure risk stratification studies.²⁸ Still, the idea at

the base of our ICD_Controls versus ICD_Cases classification is that ICD implantation problem is not to prevent sudden cardiac death alone, but also to select those patients who will benefit from ICD implantation, either by preventing sudden cardiac death (shock therapy) or by treating serious ventricular arrhythmias (antitachycardia pacing).

ECG recordings were performed during a bicycle ergometer test which included an exercise phase characterized by an increasing HR driven by an increasing workload, and a recovery phase characterized by a decreasing HR driven by a decreasing workload. TWA was quantified using our AMF²⁴⁻²⁷ which, by hypothesizing TWA as a narrow-band rather than a single-frequency phenomenon (as done by other techniques^{10,22}), is particularly

Figure 1. Plots of HR during the bicycle ergometer test and measured TWA as functions of time relative to two ICD patients for whom the end of the exercise phase (i.e., the instant at which HR was maximum) occurred at HR equal to 125 bpm (Patient A, panels a and b) and at HR equal to 140 bpm (Patient B, panels c and d), respectively. TWA peaks occurred in correspondence of the exercise-recovery transition in the first case, and in the middle of exercise in the second.

suitable to analyze ECG tracings affected by a certain amount of HR variability,^{25,29,30} as those in the present study.

Independently from the used technique, TWA identification is reliable only if performed in good quality (low noise level and limited HR variability) ECG tracings.^{24,25,29} The ECGs used here consisted of routine clinical data finalized to maximum workload assessment and were not specifically recorded for TWA analysis: neither technology nor measurement protocol was TWA-specific. To minimize the number of rejections TWA was not identified from the entire ECG recording. Rather, ECG windows of 64 consecutive beats were recursively extracted every 2 seconds and eventually submitted to our AMF for TWA identification. The window length represents a tradeoff between acceptable HR stability (short windows) and AMF stability (long windows). Instead, the recursive window extraction, which implies a lot of overlapping between a window

and the next one, was conceived to isolate possible local artifacts. TWA was not measured in poor quality (too high noise level or HR variability) ECG windows. Since each window was characterized by its own HR, for each patient TWA measurements could be available for some HRs but not for others. Moreover, since the HR range covered during the ergometer test was patient-dependent, the number of patients with detected TWA at a specific HR was strongly varying (Table 2).

According to our results, exercise TWA remained low and practically constant until HR reached 100–110 bpm and then it started to increase significantly. This finding is in agreement with results from other studies,^{10,31} and explains why most studies are performed at high HRs, typically 120–125 bpm, when TWA is significantly higher and thus easier to be detected. Low amplitude TWA indeed, may be very difficult to identify, especially when threshold-based methods for TWA identification are used.³² Such methods ascribe low

Figure 2. Plots of mean TWA as function of HR during the exercise (arrows toward increasing HR) and recovery (arrows toward decreasing HR) phases of the ergometer test relative to the entire ICD population (a), the ICD_Cases (b), and the ICD_Controls (c). Asterisks (*) indicate HRs for which exercise and recovery TWA significantly differed.

levels of TWA (i.e., TWA levels under threshold) to noise and hypothesize TWA to be an on-off phenomenon, not present or present with over-threshold amplitudes. Instead, having our AMF being designed on the theoretical assumption of TWA being a continuous phenomenon whose amplitude may continuously vary from zero to positive values, its algorithm does not include a minimum cutoff level over which TWA is identified. The assumption of TWA being a continuously varying phenomenon derives from the observation of rare cases of visible TWA that show how the phenomenon is substantially nonstationary, and may gradually change its amplitude, going from visible to not-visible (potentially absent), and from concordant to discordant.³³ Both visible and microvolt forms of TWA are identified by the AMF, which is able to quantify but not distinguish between concordant versus discordant TWA.

Thanks to its features, the AMF is suitable to identify also low levels of TWA such as those characterizing the ICD patients at the beginning of the exercise test. The HR at which TWA started

to increase in this study matches the HR that in the literature is called TWA-onset HR.^{10,34} After, TWA kept increasing till HR reached 120–125 bpm. Eventually, for higher HRs, TWA declined to its initial values. The cause of this loss of TWA at high HR, earlier observed by Bloomfield et al,¹⁰ is still not known. This phenomenon, however, implies that the peak of alternans may be reached either during exercise or at the end of it (i.e., at the instant of MaxHR and workload; Fig. 1) depending on the patient HR reaction to exercise. During recovery, when HR returned to values around 120–135 bpm, TWA also increased but in a much less marked way than during exercise. Thus, TWA showed a HR-dependent hysteresis, since its value did not only depend on HR actual value but also on such HR being reached during exercise or recovery (Fig. 2). Consequently, HR and TWA are not linked by a one-to-one correspondence, and studies involving TWA measures at a specific HR should take into account how such HR has been reached. An analogous finding was previously reported in studies which have compared TWA

Table 2. TWA (Median [25th; 75th Percentiles]) at Various Heart Rates (HR) during Both Exercise and Recovery Phases of the Bicycle Ergometer Test

HR (bpm)	ICD (266)						ICD_Cases (76)						ICD_Controls (190)					
	Exercise			Recovery			Exercise			Recovery			Exercise			Recovery		
	TWA (μV)	# pts		TWA (μV)	# pts		TWA (μV)	# pts		TWA (μV)	# pts		TWA (μV)	# pts		TWA (μV)	# pts	
70	17 [15;24]	51		17 [12;31]	22		23 [14;27]	11		17 [12;25]	4		17 [15;22]	40		17 [12;33]	18	
75	17 [12;24]	97		20* [14;31]	49		16 [12;26]	21		19 [15;27]	11		17 [13;24]	76		23* [15;32]	38	
80	16 [12;25]	137		22* [15;32]	83		20§ [13;31]	36		27 [18;39]	19		15 [12;22]	101		21* [13;29]	64	
85	18 [12;25]	169		22* [14;35]	99		20 [14;28]	45		29 [14;50]	25		17 [12;25]	124		21* [15;34]	74	
90	16 [12;23]	201		23* [14;41]	120		17 [13;24]	54		38†§ [17;49]	34		16 [12;23]	147		21* [14;36]	86	
95	17 [12;25]	209		25† [16;37]	147		18 [13;28]	55		33†§ [17;46]	38		17 [12;25]	154		24† [16;33]	109	
100	18 [12;26]	217		26† [18;41]	181		19 [13;29]	60		27* [18;44]	45		17 [12;24]	157		25† [18;38]	136	
105	19 [13;29]	211		25† [17;42]	180		20 [12;30]	56		24* [19;46]	45		19 [13;29]	155		26† [17;42]	135	
110	21 [14;35]	181		27† [18;42]	148		21 [14;32]	50		28* [20;42]	37		21 [15;35]	131		27* [18;42]	111	
115	28 [18;50]	153		27 [17;42]	127		24 [17;49]	38		31 [19;54]	28		29 [18;50]	115		26 [17;39]	99	
120	41 [27;69]	137		28† [20;40]	113		38 [24;66]	30		31 [21;60]	23		41 [28;72]	107		27† [19;37]	90	
125	51 [32;81]	113		28† [20;38]	94		38 [29;77]	26		30 [24;49]	21		53 [34;84]	87		27† [18;36]	73	
130	41 [26;67]	94		28† [18;41]	69		35 [17;63]	23		28 [18;57]	17		41 [29;68]	71		28† [17;40]	52	
135	29 [19;54]	67		26 [15;40]	52		20 [14;56]	13		26 [17;42]	12		30 [23;54]	54		25* [15;40]	40	
140	22 [16;82]	54		22 [14;37]	48		15§ [11;24]	12		24 [15;41]	12		22 [18;33]	42		21 [13;37]	36	
145	19 [14;31]	36		18 [14;27]	37		18 [14;23]	8		18 [14;30]	9		19 [14;35]	28		19 [14;27]	28	
150	22 [15;27]	25		20 [15;24]	23		19 [10;23]	5		20 [15;53]	5		22 [16;30]	20		21 [15;24]	18	

* †, ‡, and §: $P < 0.05$, $P < 10^{-3}$, and $P < 10^{-6}$, respectively, when comparing exercise versus recovery TWA at a specific HR within a group.§: $P < 0.05$ when comparing ICD_Cases versus ICD_Controls TWA at a specific HR within a test phase.

at various HRs reached through exercise versus pacing, rather than exercise versus recovery.^{31,34-37} Such studies have observed that TWA levels measured during exercise and pacing are not comparable and thus concluded that there is low concordance rate between test results using different protocols. Narajan et al.³¹ hypothesized that progressive changes in HR may produce hysteresis in TWA, and looked for a definition of an optimal HR dynamics for applying TWA to risk stratification for inducible VT. The study was performed in patients undergoing programmed electrical stimulation of the ventricle. The results indicated that TWA was sensitive to the direction of HR change (i.e., there was a TWA hysteresis on HR) as well as to its absolute value and that optimal separation of inducible from noninducible VT was during acceleration at 120 bpm. When comparing the results of this study with those obtained in the present work, it can be observed that in both cases the highest TWA occurs during acceleration at about 120–125 bpm. However, in our case such high HR does not represent the optimal HR for discriminating ICD_Cases from ICD_Control, that is for predicting VT/VF, which is instead found at lower HR (80 bpm during exercise, and 90–95 bpm during recovery).

Overall, in our ICD patients exercise TWA and recovery TWA were, on average, comparable. However, given the different way TWA changed with HR during the two phases of the test, TWA resulted statistically lower during exercise than during recovery for HR between 75 and 110 bpm, and reverse for HR between 120 and 130 bpm. The highest TWA levels were observed during exercise for HR = 120–130 bpm, rates at which risk stratification studies on exercise TWA are usually performed.¹⁰ Still, such HR range was not optimal to discriminate ICD_Cases from ICD_Controls (Table 2), that is to identify those ICD patients who developed VT/VF during follow-up, and thus who were retrospectively identified as at increased risk of major cardiac events. Similarly to what found in our previous studies,⁷⁻⁹ the best exercise HR for discriminating the two ICD groups was 80 bpm, in correspondence of which ICD_Cases and ICD_Controls were characterized by low but significantly different TWA levels, with the former showing higher TWA than the latter (Table 2). Such HR also represents a common HR at which arrhythmias occur,^{38,39} observation that supports the hypothesis of TWA being an

arrhythmogenic phenomenon. Another exercise HR in correspondence of which TWA was significantly different between the two ICD groups was 140 bpm (after dropping down from its maximum values) for which, however, TWA was lower in ICD_Cases than ICD_Controls (Table 2). Still, only some ICD patients reached such HR during exercise (16% of ICD_Cases and 22% of ICD_Controls), and thus it should not be considered for routine TWA testing. During recovery, TWA was significantly greater in ICD_Cases than ICD_Controls for HR = 90 bpm and HR = 95 bpm (Table 2), indicating that the recovery phase of an ergometer test can provide additional information to improve identification of patients at increased risk of arrhythmic events. It has been observed that, beyond TWA, impairment of HR recovery, as a reflection of decreased vagal reactivation, is directly related to increased risk of cardiovascular events.⁴⁰ Thus, further studies will investigate how HR recovery impacts TWA hysteresis on HR. So far, neither exercise nor recovery TWA has shown a sufficient discrimination power to justify prognosis evaluations based only on it. Rather, clinical utility of TWA could be enhanced if exercise TWA as well as recovery TWA are combined, possibly also with other parameters.^{5,41}

Finally, it might be argued that the two TWA patterns in Figure 1 might be related to patient's heart condition. High MaxHR are required to observe the initial TWA increment followed by the later decrement at higher HRs, and it might be expected that patients with low LVEF cannot reach such high HRs. Indeed, ICD_Cases were characterized by lower LVEF than ICD_Controls (Table 1) indicating a worse condition of the heart and, thus, a higher propensity to VT/VF. However, MaxHR was comparable in the two ICD groups, and thus has no discrimination power. Consequently, MaxHR cannot have caused the observed TWA hysteresis differences.

In conclusion, in our primary prevention ICD patients, TWA shows a HR-dependent hysteresis, since its value does not only depend on HR actual value but also on such HR being reached during exercise or recovery. A different behavior of TWA is observed in ICD_Cases and ICD_Controls. Consequently, beside exercise TWA also recovery TWA may contribute to identify subjects at increased risk of arrhythmic events.

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