Adrenergic stimulation promotes T-wave alternans and arrhythmia inducibility in a TNF-α genetic mouse model of congestive heart failure

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Shusterman V, McTiernan CF, Goldberg A, Saba S, Salama G, London B. Adrenergic stimulation promotes T-wave alternans and arrhythmia inducibility in a TNF-α genetic mouse model of congestive heart failure. Am J Physiol Heart Circ Physiol 298: H440–H450, 2010. First published November 25, 2009; doi:10.1152/ajpheart.01024.2008.—T-wave alternans (TWA) is a proarrhythmic repolarization instability that is common in congestive heart failure (CHF). Although transgenic mice are commonly used to study the mechanisms of arrhythmogenesis in CHF, little is known about the dynamics of TWA in these species. We hypothesized that TWA is present in a TNF-α model of CHF and can be further promoted by adrenergic stimulation. We studied 16 TNF-α mice and 12 FVB controls using 1) in vivo intracardiac electrophysiological testing and 2) ambulatory telemetry during 30 min before and after an intraperitoneal injection of isoproterenol. TWA was examined using both linear and nonlinear filtering applied in the time domain. In addition, changes in the mean amplitude of the T wave and area under the T wave were computed. During intracardiac electrophysiological testing, none of the animals had TWA or inducible arrhythmias before the injection of isoproterenol. After the injection, sustained TWA and inducible ventricular arrhythmias were observed in TNF-α mice but not in FVB mice. In ambulatory telemetry, before the isoproterenol injection, the cardiac cycle length (CL) was longer in TNF-α mice than in FVB mice (98 ± 9 and 88 ± 3 ms, P = 0.04). After the injection of isoproterenol, the CL became 8% and 6% shorter in TNF-α and FVB mice (P < 10−4); however, the 2% difference between the groups in the magnitude of CL changes was not significant. In TNF-α mice, the magnitude of TWA was 1.5–2 times greater than in FVB mice both before and after the isoproterenol injection. The magnitude of TWA increased significantly after the isoproterenol injection compared with the baseline in TNF-α mice (P = 0.003) but not in FVB mice. The mean amplitude of the T wave and area under the T wave increased 60% and 80% in FVB mice (P = 0.006 and 0.009) but not in TNF-α mice. In conclusion, TWA is present in a TNF-α model of CHF and can be further promoted by adrenergic stimulation, along with the enhanced susceptibility for ventricular arrhythmias.

Tumor necrosis factor-α model; ambulatory telemetry

During the last decade, a number of investigations have been devoted to the detailed analysis of the spatial distribution and short-term temporal dynamics of TWA using electrical and optical mapping techniques (10, 13, 32). However, the temporal evolution of TWA in a real-life setting has only recently become a focus of intensive research (28, 36, 43, 44). A broad spectrum of temporal variations in the magnitude of TWA has been described in ambulatory Holter recordings obtained from human subjects. In particular, an upsurge in the magnitude of TWA has been observed during the periods of elevated sympathetic nervous system activity (17, 43). Since the sympathetic activity and TWA are also increased before the onset of VTAs (37, 38), analysis of the links between the autonomic activity and repolarization instability is important for an understanding of the physiological processes involved in arrhythmogenesis.

Recent advances in genetic engineering have provided the tools for modeling of the mechanisms and pathophysiology of cardiovascular diseases. In particular, a TNF-α genetic mouse model (TNF-α mice) has been developed to investigate the pathogenesis of congestive heart failure (CHF) by overexpressing the inflammatory cytokine TNF-α in the heart (16). TNF-α mice develop biventricular dilatation, decreased ejection fraction, and atrial and ventricular arrhythmias and have increased mortality compared with littermate control mice (20, 34, 39). The majority of TNF-α mice demonstrate tachycardia, cyanosis, and ascites before death and show evidence of decompensated heart failure at autopsy (20). We (20) have previously demonstrated that Ca2+ alternans develops at longer pacing cycle lengths (CLs) in TNF-α mice than in controls using optical mapping of isolated hearts. For this reason, we hypothesized that TWA would be increased at baseline in TNF-α mice and that its magnitude would be modulated by autonomic nervous system activity.

Here, for the first time, we show that adrenergic stimulation promotes TWA in TNF-α mice using a combination of intracardiac electrophysiological testing in vivo and continuous, ambulatory telemetry ECG recording in freely moving animals. The emergence of TWA elicited by the adrenergic stimulation was associated with heightened arrhythmia vulnerability in TNF-α mice.

METHODS

All experiments were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. Heterozygous TNF-α transgenic mice engineered on a FVB background were bred with FVB controls to generate TNF-α mice [also referred to as TNF1.6 mice elsewhere (34)] and wild-type littermate controls. FVB mice were used because they are the most common strain used for overexpression studies of transgenic mice. For all experiments, TNF-α and control mice were age, sex, and strain matched. In vivo intracardiac electrophysiological testing. The relationship among adrenergic stimulation, arrhythmia inducibility, and the emer-
gence of TWA has not been studied in mice. The electrophysiological testing was performed in seven TNF-α mice and four FVB mice to examine 1) the correspondence among the presence, magnitude, and duration of TWA in intracardiac and surface ECG recordings before and after adrenergic stimulation and 2) the inducibility of ventricular arrhythmias before and after adrenergic stimulation (Fig. 1).

Animals were anesthetized, and the in vivo electrophysiological testing was performed as previously described (34). Briefly, a six-lead electrocardiogram was recorded continuously from the 24-gauge needles inserted subcutaneously in each limb. A 2-Fr octapolar catheter (NuMed, Hopkinton, NY) was advanced through the right external jugular vein to the right ventricle until a bipolar His bundle electrogram could be recorded from the middle electrodes. Baseline parameters were measured in all mice, including CL and PR, QRS, QT, AH, and HV intervals as previously described (34). Atrial and ventricular pacing thresholds were then determined, and pacing was conducted using 2.0-ms pulse widths at twice diastolic threshold. Sinus node function was evaluated by measuring the sinus node recovery time (SNRT) after the right atrium was paced at a CL of 100 ms for 60 s and correcting it for the baseline heart rate (corrected SNRT = SNRT − CL). Effective refractory periods as well as functional refractory periods were determined at a CL of 100 ms. Programmed atrial and ventricular stimulation was performed in both atria and ventricles, as we have previously described (34). The inducibility of ventricular arrhythmias was defined as 10 beats of ventricular tachycardia after the last pacing stimulus. Ten minutes after the administration of β-adrenergic stimulation [isoproterenol (2 ng/g ip)], the electrophysiological protocol was repeated as described above.

Ambulatory telemetry recordings. Ambulatory telemetry recordings were examined in eight FVB mice (age: 3–9 mo, 88% female and 12% male) and nine TNF-α transgenic mice (age: 3–9 mo, 60% female and 40% male). Radiotelemetry electrocardiographic monitors (DSI, St. Paul, MN) were implanted subcutaneously on the backs of

Fig. 1. In vivo intracardiac electrophysiological testing in a TNF-α mouse. A and B: surface ECG and two intracardiac (from the electrodes located inside the right ventricle) electrograms before (A) and after (B) the injection of isoproterenol. Pronounced, sustained (>15 min) T-wave alternans (TWA) is present in the surface ECG and in both intracardiac electrograms. Averaged and superimposed even and odd beats (C) show that the alternans occurred during the repolarization phase (T wave), whereas the depolarization phase (QRS complex) did not show any alternans. Also note that the end of the T wave in the surface ECG occurred simultaneously with the end of the repolarization phase in the intracardiac recordings. D: emergence of sustained, spatially widespread (registered by all 4 electrodes located inside the right ventricle) alternans after the injection of isoproterenol was associated with heightened arrhythmia vulnerability. Multiple, rapid, polymorphic ventricular tachyarrhythmias (VTAs) could be induced by programmed stimulation from the intraventricular electrodes during 45 min after the injection. In contrast, no arrhythmias could be induced and no alternans were observed before the injection.
the mice under tribromoethanol (Avertin) anesthesia as previously described (34). Mice were allowed to recover for at least 6 days. Intraperitoneal injections of isoproterenol (1 µg) were performed at the same time of day and in the same room.

Analysis of cardiac repolarization. Examples of ECG data recorded from TNF-α and FVB mice before and after the injection of isoproterenol are shown in Fig. 2. ECG data were digitized at 400 Hz and 16-bit resolution and interpolated to 1,600 Hz using cubic spline to

Fig. 2. Raw electrocardiographic data obtained before (left) and 5 min after (right) the injection of isoproterenol in two TNF-α mice (TNF#69 and TNF#71) and a FVB mouse (FVB#87). Note that TWAs (period 2 oscillations) are visible in TNF#69 and TNF#71 after the injection of isoproterenol. In contrast, the amplitude of the T wave in FVB#87 exhibited relatively slow (period 4) oscillations, associated with respiration, but no visible alternans. Arrows indicate the location of the T waves.
enhance the time resolution. Baseline drift was removed using a previously validated adaptive filtering approach that accurately detects an isoelectric line with a minimal distortion of the repolarization waveforms (40). In short, the method consists of two steps: 1) high-pass filtering of those segments where the level of baseline wander is greater than a threshold and 2) a high-fidelity estimation of the isoelectric level using a linear interpolation between the PQ and TP segments and subtraction of the residual error (40). This procedure avoids excessive filtering and signal distortion by 1) eliminating the high-pass filtering of relatively “clean” segments with small baseline wander [only the second step of the correction procedure is applied to such “clean” segments, which constitute $\approx 75\%$ of the entire recording time (40)] and 2) adapting the filtering cutoff to the frequency of baseline drift in the rest of the segments, where the drift is relatively large and the filtering is unavoidable. Since the lowest frequency components of the cardiac complexes in 99% of adults 99% of the time are $>0.67$ Hz, the upper limit for the cutoff frequency of the high-pass filter is 0.67 Hz, as recommended by the American Heart Association and EC38:1998 Standard for Ambulatory Electrocardiographs (3, 4). Note that the ST segment may include frequency components as low as 0.05 Hz and that the high-pass filtering may affect the slope of the ST segment. However, such systematic changes would not affect the level of TWA (i.e., beat-to-beat alterations) because all beats would have the same amount of attenuation in the ultra-low-frequency range (0.05–0.67 Hz).

The baseline correction procedure described above has been shown to provide more accurate estimation of the isoelectric level and smaller ECG distortion than high-pass filtering (40).

QRS complexes were classified and fiducial points, including the J-point, the peak of the T wave, and end of the repolarization segment, were detected using validated software and verified by an experienced Holter technician (36, 40). The onset of repolarization in mice is not clearly separated from the QRS complex. Therefore, our analysis did not include the earliest part of repolarization that is masked by the QRS. The onset of the T wave was determined by an abrupt decrease of the first derivative of the signal, marking the end of QRS complex (Fig. 3A).

Because a single, similarly positioned lead was used in each animal for all analyses in all time intervals, interlead differences in T-wave amplitudes would not be expected to affect the results (8). Since the changes in repolarization are complex and highly variable among individual animals and no single parameter on the surface ECG can reliably represent the entire spectrum of repolarization changes (23), we used a set of several descriptors in addition to TWA, including the duration of the QT interval, mean T-wave amplitude, and T-wave area, as previously described (41).

Artifact and residual baseline drift control. To exclude possible spurious artifacts superimposed on the repolarization segment, each T wave was compared with the average of all T waves in the corresponding 37.5-s interval (referred to as the average T wave) using a linear cross-correlation. If the correlation coefficient was $<0.85$ or the peak amplitude of the T wave was more than two times greater than that in the average T wave, the waveform was excluded from analysis. Residual baseline instabilities were measured over the isoelectric TP segment as described elsewhere (36). If the amplitude of baseline instability exceeded 50 $\mu$V or its SD was $>25$ $\mu$V, those beats were also excluded (28). Data segments with uninterrupted streams of consecutive T waves were used for TWA analysis.

One electrocardiographic recording from a TNF-α mouse was excluded from analysis because the T waves were undetectable. In the recordings that were included in the analysis, there were $<6\%$ excluded beats due to all causes combined (i.e., ventricular ectopy: $<1\%$ and artifacts, muscle noise, and baseline wander: $<5\%$).

Selection of time windows for analysis. We found that the mean heart rate in control mice ($645 \pm 48$ beats/min) was approximately eight times higher than in humans assuming an average heart rate in healthy humans of 80 ± 7 beats/min (34, 35, 39). Therefore, the time intervals were adjusted for these high heart rates. The duration of the time intervals for analysis was obtained by dividing those traditionally used in humans (300- and 3,600-s intervals) by 8. The time course of changes in CL and repolarization variables during the injections of isoproterenol was analyzed in 37.5-s (300/8) intervals and averaged for each consecutive 7.5-min interval (60/8), as shown in Fig. 3, C and D.

TWA analysis. TWAs were examined using two filtering approaches: 1) a linear filter [intrabeat averaging (IBA)] and 2) a nonlinear filter [modified moving average (MMA)] as described elsewhere (17, 28, 36). Since the two approaches produced similar trends in this (Figs. 3 and 4) and previous validation studies, numer-
tional results are presented for IBA only (17, 36). Although the two methods may give different numerical estimates of the absolute levels of TWA, the focus of this study was to investigate the trends (relative changes) of TWA. In short, the IBA computer algorithm consisted of 1) calculation of the mean amplitude of the following segments: from the onset of the T wave to the peak of the T wave, from the peak of the T wave to the end of the T wave, and from the onset of the T wave to the end of the T wave; 2) calculation of the time series of the differences between the mean amplitudes of consecutive even and odd beats in each segment; and 3) averaging of these time series over 37.5-s intervals.

Statistical analysis. Shapiro-Wilks’ W-test of normality was used to assess the distribution of the data. Because of substantial deviation from the normal distribution, nonparametric Friedman ANOVA for repeated measurements was applied to test the significance of changes in each variable over time. If overall changes were significant by the Friedman ANOVA, a Wilcoxon test was used to determine which data points were significantly different from the baseline reference periods. To estimate the effects of isoproterenol, a 7.5-min (baseline) window of the myocardium was selected 7.5–15 min before the test. Similarly, before and six consecutive 7.5-min windows after the injection were included in the nonparametric ANOVA. To exclude the effects of artifacts and ambient noises caused by preparation for the injection, the baseline period was selected 7.5–15 min before the test. Similarly, the 5-min period immediately after the injection was excluded from the analysis due to the high level of artifacts and noise. Comparisons between the TNF-α and FVB groups were performed using the Mann-Whitney U-test. Results are presented as means ± SD unless indicated otherwise. Statistical significance was accepted at the level of P < 0.05.

RESULTS

In vivo intracardiac electrophysiological test. Figure 1 shows examples of sustained (>10 min), macroscopic (>100 μV) TWA that emerged in a TNF-α mouse after the injection of isoproterenol and were identifiable by visual inspection in all four intraventricular and six surface ECG leads. Note also that averaging and superimposing even and odd beats (Fig. 1C) showed that the alternans occurred only within the repolarization segment, whereas the amplitude of the depolarization segment (QRS complex) did not show any beat-to-beat alternans. Importantly, the alternans and the end of repolarization segment in the intracardiac and surface ECG occurred simultaneously, confirming the validity of tracking repolarization alternans in extracardiac data (surface ECG or telemetry recorded from the subcutaneously implanted electrodes). However, the onset of alternans in the surface recordings was masked by the overlapping QRS complex and could not be accurately analyzed in the extracardiac data (Fig. 1C).

The emergence of sustained, macroscopic alternans in all recorded intraventricular leads was associated with heightened arrhythmia vulnerability (Fig. 1D). Before the injection of isoproterenol, no arrhythmias could be induced by programmed stimulation in either TNF-α or FVB mice. In contrast, multiple (>10 episodes), polymorphic VTAs could be induced by the same stimulation protocol 10–30 min after the injection in TNF-α mice but not in FVB mice (Fig. 1D).

Ambulatory telemetry recordings. Examples of ambulatory telemetry recordings obtained before and after the injection of isoproterenol are shown in Fig. 2; averaged waveforms and processing of the TWA signals are shown in Fig. 3. Below we describe the changes in cardiac CLs, TWA, and other indexes of repolarization in freely moving animals using the ECG telemetry data.

Changes in the magnitude of TWA. In TNF-α mice, the magnitude of TWA was ~1.5–2 times greater than in FVB mice both before and after the injection (Table 1 and Fig. 4). After the injection, the magnitude of TWA increased significantly compared with baseline in TNF-α mice but not in FVB mice (P = 0.003 and 0.17, respectively; Table 1). In TNF-α mice, TWA was elevated relative to baseline during ~15 min after the injection, which coincided with the greatest shortening of CL (Fig. 5). During this period, the magnitude of TWA was significantly higher in TNF-α mice compared with FVB mice. In TNF-α mice, the magnitude of TWA after the injection was increased in both the early (from the onset of the peak of the T wave) and late (from the peak to the end of the T wave) portions of the T wave (Table 1 and Fig. 6).

Changes in cardiac CLs. The time course of changes in cardiac CLs in both groups is shown in Fig. 5. Before the injection of isoproterenol, the CL was longer in TNF-α mice.
Table 1. Indexes of repolarization and cardiac cycle lengths before (baseline) and 5–12.5 min (peak effect) after the injection of isoproterenol

<table>
<thead>
<tr>
<th>Indexes</th>
<th>TNF-α Mice</th>
<th>FVB Mice</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before injection</td>
<td>After injection</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Median</td>
</tr>
<tr>
<td>TWA, μV</td>
<td>29.5 ± 19.1</td>
<td>23.0</td>
</tr>
<tr>
<td>TWAonset-peak, μV</td>
<td>35.2 ± 22.7</td>
<td>26.8</td>
</tr>
<tr>
<td>TWApeak-end, μV</td>
<td>32.4 ± 20.2</td>
<td>28.3</td>
</tr>
<tr>
<td>Mean amplitude of the T wave, mV</td>
<td>11.5 ± 10.3</td>
<td>9.7</td>
</tr>
<tr>
<td>Area of the T wave, mV ms</td>
<td>98.2 ± 92.9</td>
<td>97.0</td>
</tr>
<tr>
<td>Cycle length, ms</td>
<td>42.9 ± 7.0</td>
<td>43.8</td>
</tr>
<tr>
<td>QT interval, ms</td>
<td>43.8 ± 7.2</td>
<td>45.0</td>
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TWA, T-wave alternans; TWAonset-peak, TWA in the early (from the onset to the peak of the T wave) portion of the T wave; TWApeak-end, TWA in the late (from the peak to the end of the T wave) portion of the T wave. Values for TNF-α and FVB mice were determined by nonparametric Friedman ANOVA for repeated measurements that included a 7.5-min baseline period and six consecutive 7.5-min periods after the injection. P values for TNF-α versus FVB mice were determined by a Mann-Whitney U-test.

Discussion

Main results. The main finding of this study was an observation that TWA is increased in a TNF-α mouse model of CHF, compared with littermate controls despite the longer CL. Adrenergic stimulation promoted arrhythmia vulnerability and further increased the magnitude of TWA in TNF-α mice but not in control mice. The study also demonstrated the feasibility of tracking the dynamics of TWA in vivo in freely moving animals using implantable telemetry devices. This result could be of practical importance for studying the pathogenesis of cardiovascular diseases and, in particular, electrophysiological abnormalities in genetic mouse models of CHF. The existence of TWA at genetic mouse model of CHF. The previous observations of electrophysiological abnormalities in this model have been considered as a tool for studying the pathogenesis of cardiovascular diseases and, in particular, electrophysiological abnormalities in genetic mouse models of CHF. The previous observations of electrophysiological abnormalities in this model have been considered as a tool for studying the pathogenesis of cardiovascular diseases and, in particular, electrophysiological abnormalities in genetic mouse models of CHF.
Fig. 6. Changes in the magnitude of TWA in the early (from the onset to the peak of the T wave \( T_{\text{onset-peak}} \); left) and late (from the peak to the end of the T wave \( T_{\text{peak-end}} \); right) portion of the T wave in TNF-\( \alpha \) and FVB mice during the injection of isoproterenol. \( T_{\text{onset-peak}} \) increased after the injection in TNF-\( \alpha \) mice but not in FVB mice \( (P = 0.004 \) and 0.11 by Friedman ANOVA for repeated measurements). Similarly, the magnitude of \( T_{\text{peak-end}} \) increased significantly after the injection in TNF-\( \alpha \) mice but not in FVB mice \( (P = 0.01 \) and 0.19 by Friedman ANOVA for repeated measurements). See Fig. 4 for details.

Fig. 7. Time course of the changes in the mean magnitude of the T wave in TNF-\( \alpha \) and FVB mice during the injection of isoproterenol. The mean magnitude of the T wave increased after the injection in FVB mice but did not change significantly in TNF-\( \alpha \) mice \( (P = 0.006 \) and 0.36 by Friedman ANOVA for repeated measurements). See Fig. 4 for details.
ure have also revealed a diminished responsiveness of the sinus node to adrenergic modulation (31).

Although we did not measure QRS alternans, our previous optical mapping study (20) in TNF-α mice has shown that the conduction velocity in these animals becomes compromised at much shorter CLs (<80 ms) than those observed in the present study. Thus, our previous observations suggest that the TWA in our study primarily represents repolarization alternans un-related to QRS alternans. This notion is further supported by our data from in vivo electrophysiological testing (Fig. 1C), which showed alternans in the intracardiac and surface ECG recordings only during the repolarization phase. However, our data cannot resolve whether the depolarization conduction slowing contributes to alternans. Moreover, the onset of repolarization in mice is masked by the QRS complex, which makes analysis of the early repolarization phase more difficult in mice that in larger animals.

There were no significant changes in the QT intervals after the injection of isoproterenol in either group, and the difference in QT intervals between the groups was also not significant. However, in our previous study (20) of TNF-α mice using optical mapping of isolated, Langendorff-perfused hearts, the action potential duration was modestly prolonged in TNF-α mice compared with wild-type mice. Furthermore, isoproterenol has been shown to increase the steepness of the action potential duration restitution slope (9). Since our measurements may not be sufficiently sensitive to capture the final, low-amplitude tail of the T wave, we cannot completely rule out the possibility of small changes in action potential duration restitution induced by isoproterenol, which could contribute to the emergence of TWA. Nevertheless, our main finding of TWA at slow heart rates in TNF-α mice is consistent with our previous optical mapping study (20) described above. In addition, the lack of changes in the amplitude and area of the T wave after the injection of isoproterenol in TNF-α mice in our present study is also in line with a similar lack of changes in action potential amplitudes in TNF-α mice at similar CLs (85–100 ms) in our previous optical mapping experiments (20).

The relatively small differences between TNF-α and FVB mice in the magnitude of TWA (the median difference between the groups after isoproterenol = 7 μV) could be related to the modest response of the heart rate to isoproterenol (<10% in TNF-α mice). Furthermore, our measurements could underestimate the magnitude of TWA because the position and vector of the recording electrodes was not optimized for measuring TWA, whose magnitude usually exhibits substantial spatial differences (24). In addition, the IBA-based estimation of TWA used in our study allows the reliable tracking of serial changes in repolarization instability but may underestimate its absolute magnitude. The latter shortcoming would not affect our main results, since the focus of our study was on the dynamics of TWA elicited by adrenergic stimulation. Furthermore, the magnitude of TWA in ambulatory telemetry recordings at baseline, despite the longer CL, was greater in TNF-α mice than in littermate controls, and adrenergic stimulation increased the level of TWA in TNF-α mice but not in control mice.

A previous study (27) conducted in humans during pacing stimulation has shown that TWA occurred later (toward the end of the T wave) in patients with inducible VTAs compared with those without arrhythmias. Presumably, the final portion of the T wave is associated with the trailing edge of repolarization and may coincide with transmural gradients, whose alternans may lead to a spatially inhomogeneous distribution of refractory periods, predisposing to irregular excitation and reentry (27). Thus, the more robust differences with respect to the magnitude of TWA in the second half of the T wave (compared with the first half) between TNF-α and FVB mice suggest that the observed alternans might be also associated with proarrhythmic gradients of repolarization. Further research is required to test this hypothesis.

**Changes in the mean magnitude and area under the T wave.**

The mean amplitude and area under the T wave increased in FVB mice but did not change in TNF-α mice. Because the data were collected using single-lead telemetry devices, analysis of the spatial distribution of these changes on the cardiac surface was not feasible. Nevertheless, it is important to note that a similar disparity between the patterns of changes in these repolarization indexes in response to the sympathetic stimulation has been observed in patients with structural heart disease compared with those without it (41).

Recent studies (1, 5) in humans with heart failure have shown that changes in the T wave amplitude and area could be related to alternations in the repolarization sequence. Thus, the changes in the T wave amplitude and area observed in FVB mice could also be related to the alterations of the repolarization sequence induced by adrenergic stimulation and associated changes in CLs. In contrast, the lack of changes in these parameters in both humans with heart failure and TNF-α mice might reflect a diminished responsiveness to adrenergic stimulation due to the underlying exhaustion of repolarization reserve or chronically high levels of sympathetic activity accompanying the development of heart failure (37, 41). Since these patterns of repolarization dynamics were similar in TNF-α mice compared with those observed in humans with chronic heart disease, our results provide further evidence of electrophysiological similarities between this genetic mouse model and the chronic heart failure phenotype in humans.

The physiological mechanisms responsible for the discordance between changes in TWA and the lack of changes in the T-wave amplitude in TNF-α mice are unknown. The results of previous investigations into this relationship in humans with heart failure have been inconsistent (1, 7). Thus, further research is needed to elucidate the relationship between TWA and other indexes of repolarization, including the T-wave amplitude and area.

**Autonomic nervous system activity and repolarization instability.** Several lines of evidence have linked autonomic activity, TWA, and arrhythmogenesis. Changes in the autonomic nervous system activity before the onset of VTA have been well documented. We (37) have previously reported increased sympathetic activity manifested by increased heart rate and changes in the spectral power of heart rate variability preceding the spontaneous initiation of VTA in human subjects with impaired ejection fraction and structural heart disease. These changes were accompanied by an upsurge in the level of TWA and nonalternating repolarization instabilities (38). Circadian changes in the frequency of VTA have provided further evidence of the participation of the autonomic nervous system in the initiation of arrhythmia (19, 37). A similar pattern of circadian changes in TWA has been observed in a postmyocardial infarction population (43). Furthermore, mental stress
has been shown to increase the level of TWA in patients with chronic heart disease (15, 17). Direct modification of sympathetic activity has also been demonstrated to alter the magnitude of TWA in a canine model of acute ischemia (29).

The mechanisms by which the autonomic activity modifies the level of TWA and, ultimately, leads to the initiation of ventricular arrhythmia are poorly understood. Although several associative and mechanistic links between autonomic activity and TWA have been previously described, our report is the first to examine the effects of adrenergic stimulation on TWA in freely moving animals. Analysis of the autonomic effects on the dynamics of repolarization in a more natural, ambulatory setting may provide further insights into the understanding of complex and multifaceted mechanisms of spontaneous arrhythmogenesis.

We (34) have previously examined the changes in heart rate variability (a noninvasive indicator of autonomic effects) elicited by the injection of isoproterenol in FVB mice. The indexes modulated by sympathetic activity (low-frequency power of heart rate variability and the ratio of low-frequency to high-frequency power) increased significantly after the injection of isoproterenol compared with saline, confirming the β-adrenergic effect of isoproterenol on cardiac function in this mouse strain (34).

**Technical challenges of the analysis of TWA in ambulatory recordings.** Analysis of the microvolt-level alternation in the amplitudes of the T waves in freely moving animals is challenging due to a number of reasons. These include high-frequency artifacts, baseline drifts, and muscle noise. Therefore, the traditional analysis of TWA has been performed in a controlled, laboratory environment and at a stable heart rate. More recently, however, methods for the analysis of TWA in ambulatory recordings have been developed and successfully applied in several patient populations (17, 28, 36, 44). As a result of the rapid proliferation of various methods in this area, the comparative analysis and cross-validation of the results obtained using different TWA estimators became difficult. Addressing this problem, Martinez et al. (25) have pointed out that all methods for TWA analysis consist of the same four processing stages (preprocessing, data reduction, TWA detection, and TWA estimation) and can be grouped into two principal categories: linear and nonlinear filtering approaches. Furthermore, Martinez et al. (25) used this classification to show that all linear filtering techniques (including the spectral method and IBA) are theoretically equivalent. The differences in performance of these methods are largely due to different properties of the analysis window (type, length, and amount of overlap), the efficiency of noise reduction, and the TWA threshold detection criteria. Thus, with appropriate preprocessing and noise control procedures, all TWA methods would yield similar results. Indeed, our previous study (38) of TWA dynamics in ambulatory ECG recordings obtained from patients with VTAs showed similar trends both for spectral and nonspectral estimators, including IBA and MMA.

The application of IBA was motivated by its relative insensitivity to the precise location of the end of the T wave, which is often difficult to determine in murine telemetry data. Furthermore, to reduce the impact of noise and outliers, each T wave was compared with the average T wave (in each 37.5-s window). In addition, the stability (the mean and SD of the baseline segment) was tested in each cardiac complex before inclusion in the analysis (36). This method has been previously validated in simulated signals and ambulatory data obtained from human subjects (17, 36, 38). However, it is important to emphasize that each filtering approach can provide only partial noise control, because an “ideal” filter is not theoretically feasible (38). For example, nonlinear filters (including MMA) achieve superior performance in the presence of random noise and artifacts, sacrificing the sensitivity to phase shifts and abrupt changes in the TWA signal (36, 38). Being aware of these technical problems, we used a previously validated preprocessing and noise control algorithm, including adaptive baseline correction, control of artifacts, and residual baseline instabilities (36, 38, 40). In addition, we included a side-by-side comparison of the TWA trends obtained using linear (IBA) and nonlinear (MMA) methods to provide additional cross-validation of our main results (Fig. 4). Note that after the injection of isoproterenol, the trends of changes in TWA determined by both IBA and MMA methods were similar, confirming the validity of our findings. Nevertheless, the two methods showed slightly different patterns in the return to baseline after the injection, with a faster decline of the MMA-measured TWA compared with the IBA-measured TWA (Fig. 4). These differences could be related to the superior robustness of the MMA method to random noise or, alternatively, its underestimation of abrupt changes and phase shifts in TWA signals (36, 38).

**Limitations.** Adrenergic stimulation increased TWA and arrhythmia vulnerability during in vivo electrophysiological testing in TNF-α mice. These observations provide the first evidence linking adrenergic stimulation, TWA, and arrhythmogenesis in a genetic mouse model of heart failure. Further research in a larger animal group is needed to determine the statistical properties and reproducibility of this association. The amount of ventricular ectopy during the ambulatory telemetry recording time was small (<1%), and the followup data on the outcomes in these animals could not be collected. The cause of death in TNF-α mice is not related to VTAs but to progressive bradycardia. Additional research is needed to determine the predictive value and potential for pharmacological blockade (by inhibitors of TNF-α receptors, β-blockers, and Ca2+ channel blockers) of TWA elicited by adrenergic stimulation in this genetic mouse model of heart failure. Further impetus for this research has been provided by a recent study (18) in humans with reduced left ventricular ejection fraction and implantable devices, showing that a high level of TWA during mental stress (i.e., another form of adrenergic stimulation) is a strong predictor of VTAs.

Data were obtained from a single-lead implantable telemetry device. Therefore, regional information regarding the spatial distribution of repolarization instabilities or the presence of discordant TWA was unavailable. However, the magnitude of repolarization instability can be site specific and depends on the location of the recording electrode (44). Nevertheless, our analysis was able to detect higher TWA in TNF-α mice compared with littermate controls and could also track changes in the magnitude of TWA elicited by adrenergic stimulation. Further research into the optimal lead configuration for the analysis of repolarization instability in murine telemetry recordings might be warranted. Studies are also needed to determine whether repolarization instability is confined to a single (TWA) range or spreads to other frequency ranges as well (38).
Conclusions. The magnitude of TWA was increased in a TNF-α model of CHF, despite the relatively long CL, and could be further promoted by adrenergic stimulation. Thus, our results suggest that the TNF-α model can be useful for the dynamical analysis of repolarization instability and its pharmacological responses. In addition, different patterns of changes in the magnitude of the T wave and its area in TNF-α and FVB mice may indicate the presence of spatial repolarization defects in TNF-α mice. Further research into the physiological mechanisms of these alterations is warranted.

Our results also suggest that a similar analysis in other transgenic and gene-targeted models may be useful in identifying the genes responsible for the expression of TWA and other types of repolarization instabilities (38).

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DISCLOSURES
V. Shusterman has a significant (>5%) ownership interest in PinMed Incorporated (Pittsburgh, PA). PinMed, Inc., provided the software used in this study. The remaining authors report no conflicts.

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