

Endurance exercise remodels pulmonary vein sleeve myocytes and promotes a proarrhythmic atrial substrate

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Abstract

Background and Aims The risk of atrial fibrillation (AF) is higher in endurance athletes. Pulmonary vein isolation (PVI) is effective in this group, implicating pulmonary vein (PV) remodelling, but underlying mechanisms are unclear. This study investigated if endurance training remodels PV sleeves and the PV–left atrial (LA) junction to promote PV triggers and a permissive peri-antral substrate for AF.

Methods In canine and murine endurance-running models, *in vivo* PV–LA mapping, *ex vivo* PV electrophysiology, intracellular action potential (AP) profiling with machine learning classification, histology, bulk RNA-seq, and subcellular-resolution spatial transcriptomics of PV–LA tissue were performed. These findings were incorporated into biophysically detailed computer models of human PV cardiomyocytes and a 3D human LA.

Results Training produced an athlete's heart phenotype and increased AF inducibility. *In vivo*, trained animals showed PV–LA conduction slowing and increased rotational activity. *Ex vivo*, trained PVs showed enhanced β -adrenergically evoked firing, prolonged burst activity, and a higher proportion of pacemaker-like APs. Spatial transcriptomics revealed discrete PV myocyte subpopulations with training upregulated *Hcn4*, *Cacna1d*, and *Cacna1g* (enhancing automaticity), downregulated *Scn5a* and *Gja1* (slowing conduction), and enriched profibrotic/inflammatory signalling (*Tnfa*, *Il6*) alongside fibroblast expansion and extracellular matrix deposition. *In silico*, these changes reproduced faster spontaneous PV firing and sustained re-entry.

Conclusions Endurance training drives coupled electrical, structural, and inflammatory PV–LA remodelling that provides both trigger and substrate for AF. These data support why PV-targeted strategies can be effective in athletic AF and nominate modifiable pathways including HCN4-linked automaticity and TNF α -associated signalling.

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Structured Graphical Abstract

Key Question

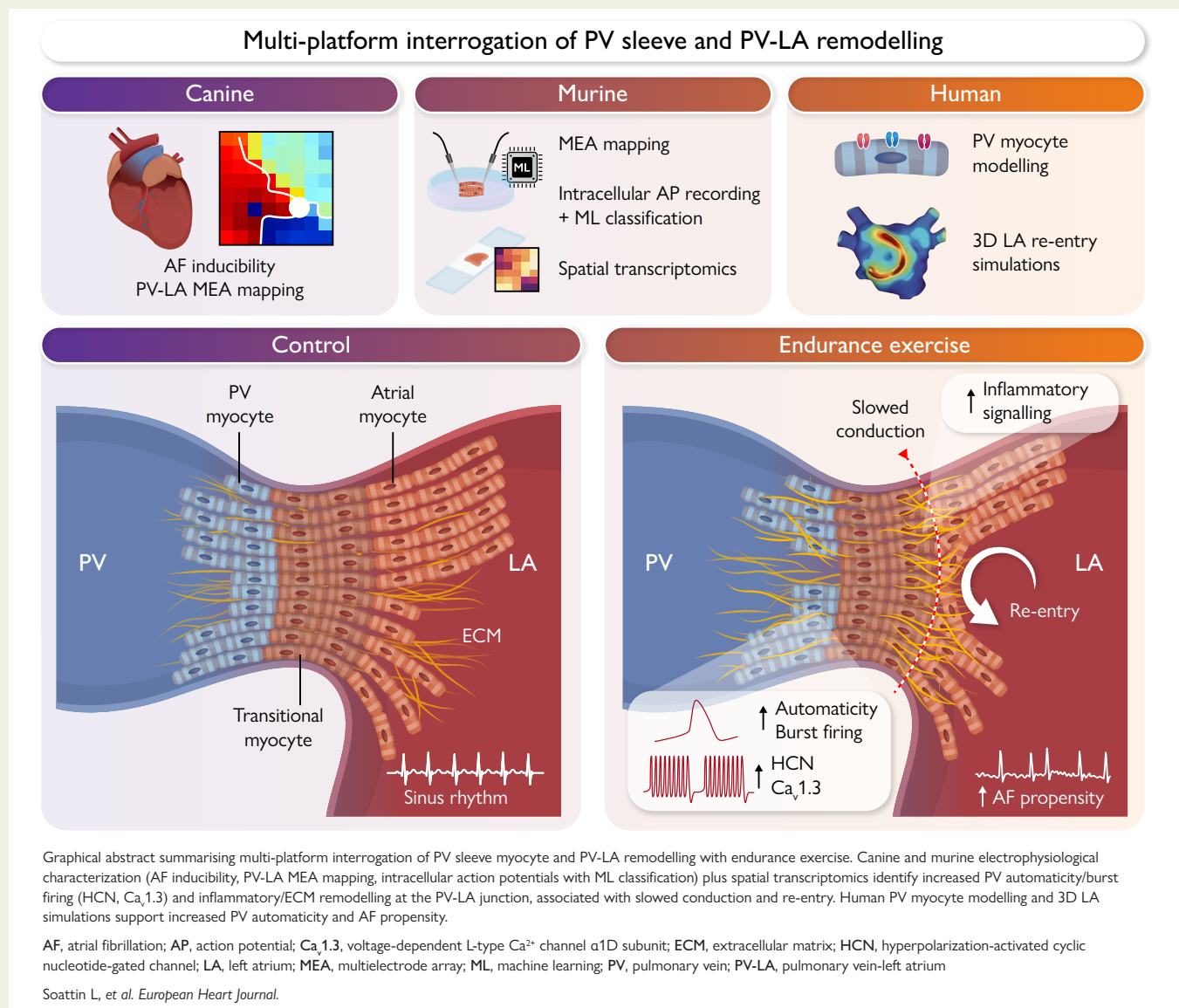
Does endurance exercise remodel pulmonary vein (PV) sleeve myocytes and the PV-left atrial (LA) junction to increase PV trigger propensity and create a permissive junctional substrate for atrial fibrillation (AF) initiation?

Key Finding

Across canine, murine and human models, endurance exercise increased PV myocyte automaticity/burst firing (HCN/ $\text{Ca}_v1.3$ -linked) and promoted inflammatory and extracellular matrix remodelling at the PV-LA junction, resulting in slowed conduction and increased susceptibility to re-entry.

Take Home Message

Endurance exercise increases AF susceptibility by heightened PV trigger propensity with PV-LA junction remodelling that lowers the threshold for conduction block and re-entry. These data provide a mechanistic basis for PV-targeted interventions in athletes at risk of AF.



Sustained high intensity endurance exercise remodels pulmonary vein sleeve myocytes and the pulmonary vein-left atrial junction.

Keywords Athletic training • Atrial fibrillation • Pulmonary vein • Ion channels • Fibrosis • Spatial transcriptomics

Translational Perspective

Endurance athletes have a paradoxically high risk of AF, yet mechanisms remain unclear. Using canine and murine models, this study demonstrates that sustained training remodels PV sleeve myocytes and the PV–LA junction, increasing pacemaker activity, slowing conduction, and activating fibrotic and inflammatory pathways. Incorporating these changes into biophysically detailed human *in silico* models reproduced increased PV trigger propensity and a lower threshold for conduction block and re-entry. These findings provide a mechanistic explanation for the clinical efficacy of PVI in athletes and highlight PV-enriched pathways as candidates for upstream prevention.

Introduction

Atrial fibrillation (AF) is increasingly recognized in long-term endurance athletes as a paradoxical complication of high levels of cardiorespiratory fitness.^{1–8} Although meta-analyses across mixed endurance cohorts suggest a modest increase in AF risk (≈ 2.5 -fold⁹) in elite lifelong endurance athletes AF burden may approach that of established high-risk groups (e.g. 21.5% in former world-class rowers vs. 3.2% in matched UK Biobank controls; prevalence risk ratio 6.8¹⁰) despite favourable traditional risk profiles. Emerging clinical evidence indicates that chronic endurance exercise can induce an atrial cardiomyopathy¹¹ characterized by atrial enlargement, mild impairment of atrial function (e.g. reduced LA strain), and mild atrial fibrosis. Work in rodent models indicates altered vagal signalling and atrial muscle remodelling, including ion-channel changes, altered intracellular Ca^{2+} handling, fibrosis, and inflammation,^{12–15} but the origin of triggers remains incompletely defined.

In clinical AF, ectopic activity from the pulmonary vein (PV) myocardial sleeves is a dominant initiator.¹⁶ Mapping studies in humans and canine preparations further indicate that the PV–left atrial (LA) junction is a critical substrate for conduction heterogeneity and re-entry, shaping both AF initiation and maintenance.^{17–19} Pulmonary vein isolation is an effective rhythm-control strategy in endurance athletes with AF,²⁰ highlighting the clinical relevance of PV triggers in this setting and motivating mechanistic investigation of training-induced remodelling within PV sleeves and the PV–LA junction. We therefore tested the hypothesis that long-term endurance training remodels the PV sleeves and PV–LA junction to increase PV trigger propensity and create a local vulnerable junctional substrate. We integrated *in vivo* and *ex vivo* electrophysiology in dogs with high-resolution cellular phenotyping, histology, and subcellular *in situ* transcriptomics in mice and incorporated the measured PV/PV–LA changes into biophysically detailed human-scale PV and atrial simulations.

Methods

Animals

Full methodological and ethical approval details are provided in the [Supplementary Material](#). The canine model enabled invasive, human-relevant *in vivo* arrhythmia susceptibility testing, autonomic pharmacology, and high-density PV–LA mapping *in situ*. Murine studies enabled high-resolution functional, molecular, and histological interrogation of PV/PV–LA remodelling (intracellular AP recording with machine-learning classification, bulk RNA-seq, and subcellular *in situ* spatial transcriptomics). All procedures were conducted in accordance with relevant national and institutional regulations.

Exercise training

Exercise training (ExT) in canines was performed as described previously.²¹ Dogs underwent a 16-week treadmill endurance programme (5 days/week), consisting each day of two 90-min continuous runs at 12–18 km/h (progressively increased) and two interval sessions (~ 50 – 60 min each) alternating 2 min at 4 km/h with 1.5 min at 22 km/h, with incline adjusted ($\sim 5\%$ – 14%). Mice underwent 6 weeks of intensity-controlled interval training alternating between 8 min at 85–90% maximum O_2 uptake ($\text{VO}_{2,\text{max}}$) and 2 min at 50–60% $\text{VO}_{2,\text{max}}$ for 60 min twice per day for 5 days per week.

Measurements

Echocardiography and ECG recordings were performed at baseline and post-training using previously established protocols.^{21–23} Canine open-chest multielectrode array (MEA) mapping, atrial monophasic action potential (MAP) recording, and arrhythmia provocation studies were conducted under terminal anaesthesia. Murine Langendorff-perfused hearts underwent programmed electrical stimulation to assess atrial arrhythmia susceptibility. Isolated canine and murine PV–LA preparations were superfused for MEA/MAP recordings. Fibrosis was assessed by Masson's trichrome staining. Mouse common PV intracellular APs were recorded using sharp microelectrodes and analysed with custom pattern-recognition software incorporating machine learning. RNA-seq and Xenium spatial transcriptomics were used to quantify differential expression, cell-type proportions, and spatial organization, with selected findings validated by immunolabelling. Biophysically detailed computer modelling was conducted using human atrial and PV models from Courtemanche *et al.*²⁴ and Dorn *et al.*²⁵

Statistical analysis

A priori power calculations (80% power, two-sided $\alpha = .05$) used atrial arrhythmia inducibility as the primary endpoint. Two-sided t-tests were used for normally distributed data, Mann–Whitney U tests for nonnormally distributed continuous data, and χ^2 test or Fisher's exact test for categorical outcomes. RNA-seq was analysed using DESeq2 with FDR correction. Xenium data were processed using Squidpy; Sed vs. ExT comparisons used the Mann–Whitney U test on pseudobulk counts with Benjamini–Hochberg correction (full detail in the [Supplementary Material](#)).

Results

Endurance training enhances AF vulnerability in canines

We previously reported on a 16-week high-intensity canine endurance training protocol that reproduces canonical, structural, and electrophysiological changes seen in human elite endurance athletes.²¹ Here, seven canines that underwent this training protocol (ExT) and seven sedentary (Sed) animals were studied.

Electrocardiogram (ECG) recordings (Figure 1A) in conscious and unrestrained animals demonstrated a significant resting bradycardia after completion of the 16-week training programme, alongside prolongation of the PQ interval, QRS duration, and the QT interval (Figure 1B). P wave duration was similar between groups (Sed, 45 ± 4 ms; ExT, 55 ± 2 ms; $P = .24$), and all ECG changes were consistent with repeated recordings in the final week of training. In line with our previous study,²¹ comparison of echocardiography parameters in ExT canines before (week 0, pre) and after (week 16, post) the training regimen confirmed an increase in left ventricular posterior wall diameter (pre, 7.6 ± 0.2 mm; post, 8.7 ± 0.4 mm; $P = .03$, paired t test) and increased left ventricular mass (pre, 64.6 ± 4.3 g; post, 77.2 ± 3.8 g; $P = .03$, paired t test) denoting training-induced left ventricular hypertrophy. The left atrium underwent structural remodelling (see Supplementary data online, Figure S1): After 16 weeks of endurance training, LA transverse diameter and area increased significantly (see Supplementary data online, Figure S1A). Increase in atrial size was also evident at the level of the cardiomyocyte which was hypertrophied in ExT atria (see Supplementary data online, Figure S1B). *In vivo* electrophysiological studies were performed to assess whether the training regimen impacted susceptibility to AF. Figure 1C shows representative surface MAP recordings from the left atrium during programmed electrical stimulation at 800 beats per minute (BPM) for 10 s. Comparison of the percentage of successful (AF-triggering) attempts out of 10 inductions (Figure 1C, right panel) demonstrated that the ExT hearts showed a higher percentage of successful AF inductions across 10 pacing trains than Sed hearts (57.1% vs. 22.9%; mean difference 34.3% points, 95% CI 2.7–65.9). Atrial fibrillation duration was also longer in ExT at baseline (3.51 vs. 1.03; mean difference 2.48, 95% CI –0.47 to 5.43; Figure 1D, left panel) and with 5 μ g/kg acetylcholine (6.10 vs. 1.85; mean difference 4.24, 95% CI 1.68–6.80, Figure 1D, right panel), consistent with greater AF vulnerability after endurance training.

Because electrical changes in athletes are often attributed to increased parasympathetic tone, we assessed autonomic contributions by measuring heart rate responses to sequential pharmacological blockade of parasympathetic and sympathetic input using atropine (0.04 mg/kg) and propranolol (0.2 mg/kg). In conscious, unrestrained dogs, the heart rate response to atropine was similar in Sed and ExT groups and unchanged pre- vs. post-training in ExT animals (Figure 1E, left panel), providing no clear evidence of a change in parasympathetic tone in this study (Figure 1E, right panel). Sympathetic tone was also comparable between groups (Figure 1F). In contrast, the intrinsic heart rate (after combined atropine and propranolol) was significantly reduced by training (see Supplementary data online, Figure S2A), consistent with a reduced spontaneous beating rate of ExT isolated (and therefore denervated) right atrial preparations in these animals (see Supplementary data online, Figure S2B), supporting training-induced intrinsic remodelling of the sinus node independent of vagal tone.^{21–23,26,27}

Endurance training modifies PV–LA conduction patterns and PV excitability in canines

High density MEA mapping of the left inferior PV and PV–LA junction *in situ* with concomitant MAP recording from the left

inferior PV and left atrial appendage (LAA) were performed to investigate PV activation and conduction patterns *in vivo* (Figure 2A). During sinus rhythm, spontaneous ectopic beats that originated in the PV and propagated to the LA were observed sporadically in ExT but not Sed hearts (Figure 2B). Representative data from an ExT heart in Figure 2B (right panel) show the direction of conduction—unlike in sinus rhythm, conduction is reversed, and the ectopic beat propagates from the PV to the LA. During programmed stimulation from the LAA (150 BPM), all ExT hearts showed lines of block at the LA–PV junction, with prolonged local activation time compared with Sed hearts (7.74 vs. 3.71 ms; mean difference 4.03 ms, 95% CI 0.11–7.94), together with altered radial propagation patterns (Figure 2C and D). Pulmonary vein AP parameters including AP duration (APD) at 90% repolarization (APD₉₀) were similar between groups (Sed, 131 ± 18 ms; ExT, 143 ± 21 ms). During triggered AF, Sed and ExT hearts exhibited distinct spatiotemporal activation patterns. Rotational activity, defined as sequential clockwise or counterclockwise isochrones around a centre of rotation, was detected on MEA recordings in 7/7 ExT and 5/7 Sed hearts (Figure 2E; a representative ExT PV rotor is shown in Figure 2E). Phase analysis of AF induced by burst pacing in the presence of 5 μ g/kg ACh showed higher rotor activity (number of rotors/total number of beats over 5 s) in ExT PVs than in Sed PVs (Figure 2E, right panel). In both groups, rotors typically persisted for at least one rotation before fragmenting into less organized wavelets. Extracellular matrix (ECM) remodelling can promote conduction heterogeneity and create pathways that support re-entry.²⁸ Consistent with a more heterogeneous re-entrant substrate, Masson's trichrome staining of PV–LA preparations showed greater ECM deposition in ExT samples (2.44% vs 1.00%; mean difference 1.44% points, 95% CI 0.98 to 1.91) (Figure 2F).

To probe intrinsic PV excitability, denervated PV–LA preparations were rapidly dissected at the end of the *in vivo* experiments, superfused, and studied with simultaneous MAP and MEA recordings after 1-h stabilization (Figure 3A). Six out of seven preparations in both groups were viable and investigated further. All were quiescent at baseline; however, β -adrenergic stimulation with 1 μ M isoproterenol elicited spontaneous APs exclusively in ExT preparations (Figure 3B). Following pacing from the right superior PV, ExT preparations showed markedly greater evoked firing than Sed preparations at both 2 Hz (3 s) and 10 Hz (3 s) in the presence of isoproterenol (Figure 3B, representative traces given in Figure 3C). In ExT preparations, episodes of evoked firing were longer in duration and exhibited a higher firing rate than in Sed preparations (Figure 3D). APD₉₀ at 2 Hz showed a trend to shortening in ExT at baseline and with isoproterenol, and APD rate dependence was greater in ExT (Figure 3E and F). Together, these *ex vivo* data show that endurance training enhances PV sleeve excitability and triggered activity.

Pulmonary vein electrophysiological remodelling in a mouse model of training-induced AF

The findings above prompted more detailed investigation of ExT-induced PV–LA remodelling in a complementary murine model of treadmill running²⁹ (full details in Supplementary

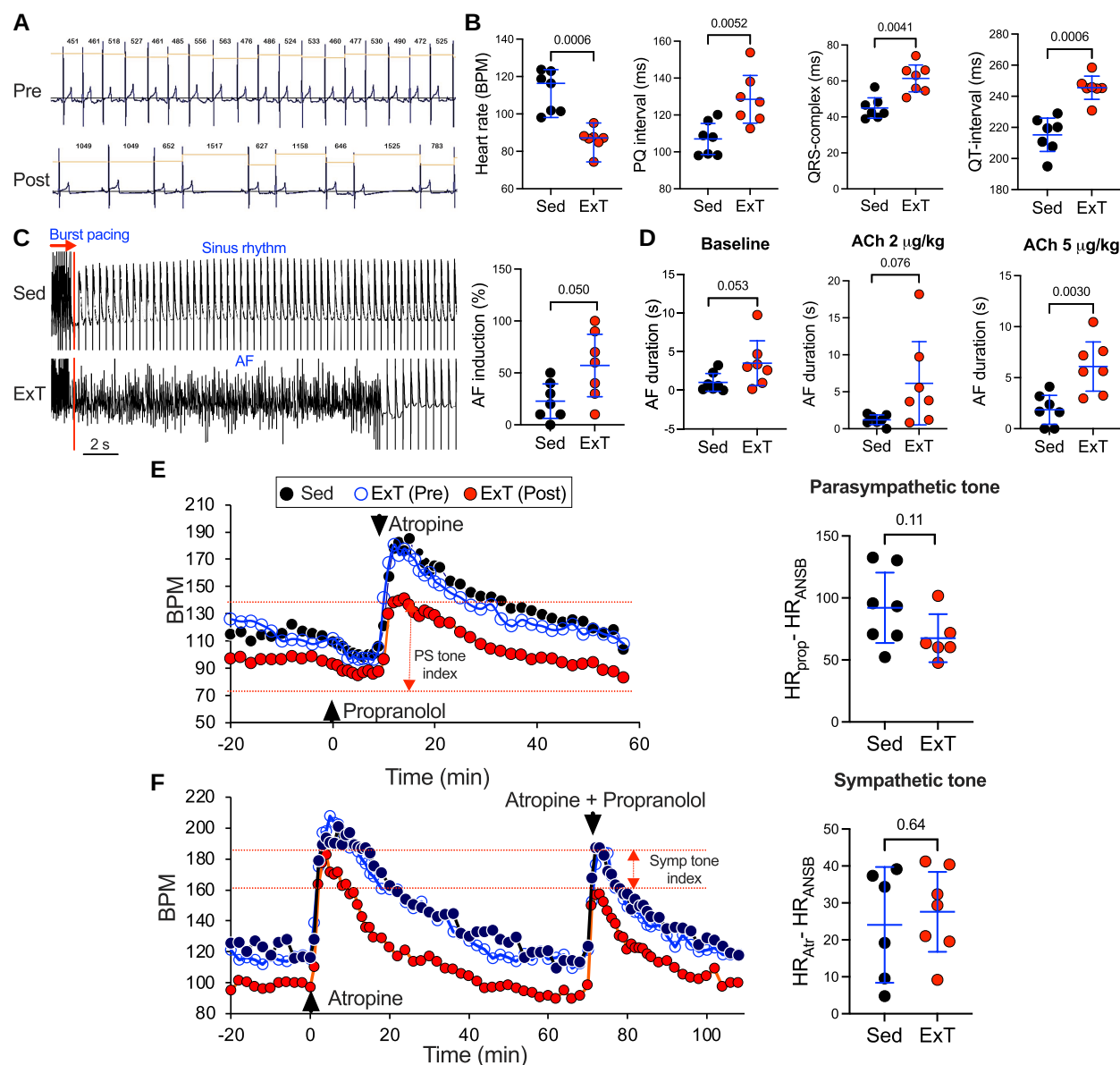


Figure 1 Athlete's heart phenotype and enhanced AF susceptibility in ExT canines. (A) Representative ECG recordings in an ExT dog prior to commencement of the training regimen (Pre) and on completion of training at week 16 (Post). (B) ECG intervals in Sed ($n = 7$) and ExT ($n = 7$) canines. (C) (left) Representative monophasic AP recordings during epicardial burst pacing (800 BPM, $3 \times$ diastolic threshold, 10 s) in Sed and ExT canines. Red line indicates cessation of burst pacing. (C) (right) AF inducibility, given as successful AF induction attempts in a total of 10 trials of burst pacing in Sed ($n = 7$) and ExT ($n = 7$) canines. (D) AF duration at baseline (left panel) and after the administration of a bolus dose of ACh delivered intravenously at 2 $\mu\text{g/kg}$ (middle panel) and 5 $\mu\text{g/kg}$ (right panel) in Sed ($n = 7$) and ExT ($n = 7$) canines. (E) (left panel) Mean heart rate recordings in conscious and unrestrained Sed ($n = 7$) and ExT (at weeks 0 and 16, pre- and post-training; $n = 7$) canines at baseline and during sequential sympathetic followed by parasympathetic block. (E) (right panel) Parasympathetic tone in Sed ($n = 7$) and ExT (at week 16 post-training; $n = 7$) canines. HR_{prop} : heart rate under sympathetic block with 0.2 mg/kg propranolol; HR_{ANSB} : heart rate under double autonomic block with 0.2 mg/kg propranolol and 0.04 mg/kg atropine. (F) (left panel) Mean heart rate recordings in conscious and unrestrained Sed ($n = 7$) and ExT (at weeks 0 and 16, pre- and post-training; $n = 7$) canines at baseline and during sequential parasympathetic followed by double autonomic block. (F) (right panel) Sympathetic tone in Sed ($n = 7$) and ExT (at week 16 post-training; $n = 7$) canines. Here and in similar graphs, points represent individual observations; horizontal lines indicate the group mean; error bars indicate 95% confidence intervals. P -values computed by Mann-Whitney U test

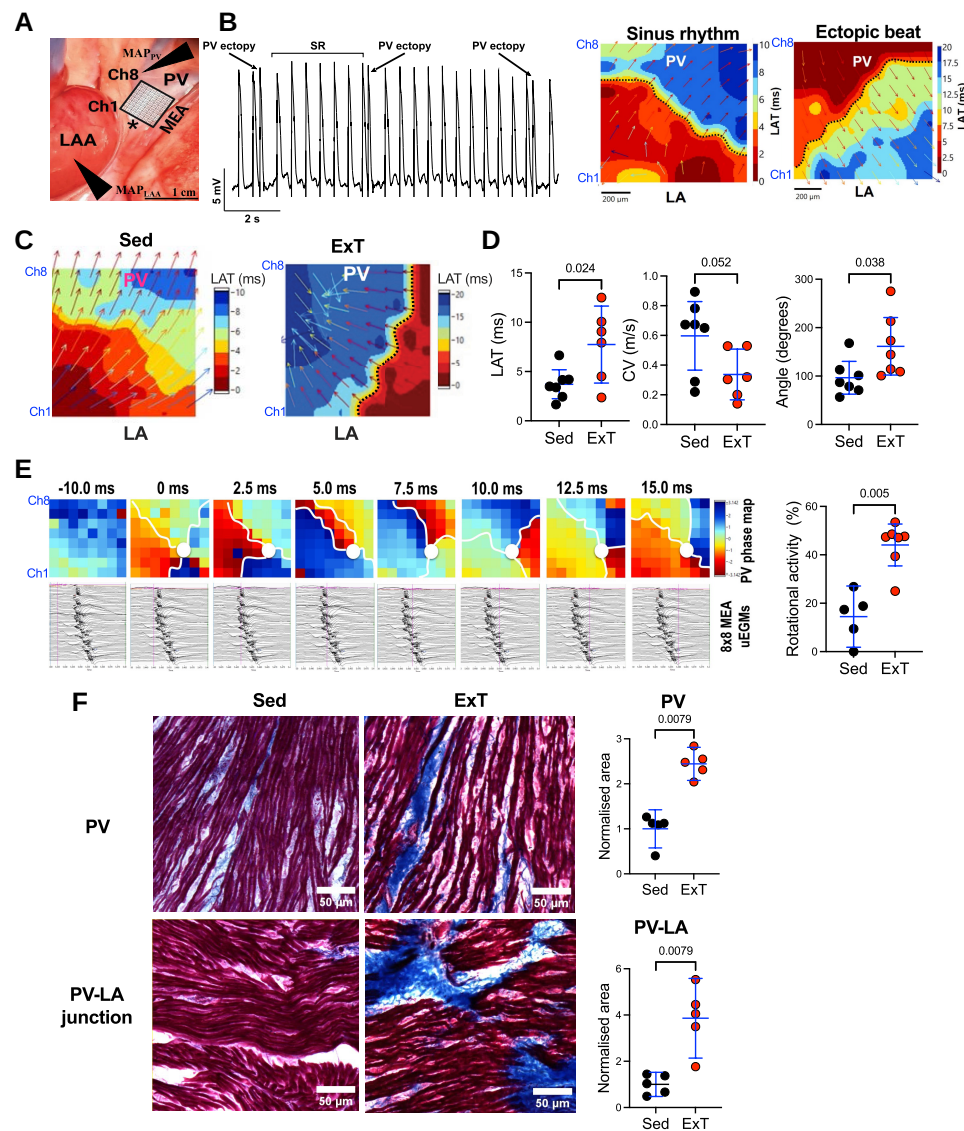


Figure 2 Exercise training results in slowed conduction and increased rotational activity in the canine PV-LA junction. (A) Placement of the 64 channel MEA on the epicardial surface of the PV-LA junction (represented by asterisk) during *in vivo* open chest experiments in canines. Channels 1 and 8 (Ch1 and Ch8) indicate the orientation of the MEA in this and in all representative maps. MAP electrodes were positioned on the LAA or the PV (arrows). (B) Representative simultaneous MAP (left panel) and MEA recordings (right panels) during normal sinus rhythm (AP propagates from the LA to the PV) and spontaneous PV ectopic beats (AP propagates from the PV to the LA). Isochronal activation maps denote local activation time (LAT), in which earliest activation is in red and latest is in blue. Arrows represent local direction of propagation. (C) Representative PV-LA isochronal maps during 2.5 Hz pacing from the LAA in Sed and ExT canines. Note isochronal crowding and prolonged LATs in ExT PV-LA. (D) Summary data from experiments like that in C denoting LAT (left), conduction velocity (CV; middle), and direction of radial propagation (right) in Sed ($n = 7$) and ExT ($n = 7$) canines. Activation maps are displayed in the same endocardial orientation as Figure 2A; dashed segments indicate defined lines of block, whereas arrows indicate local propagation. (E) (top panel) Representative recording of rotational activity in the canine PV-LA junction. Phase maps at baseline (-10 ms) and during a complete rotational reentrant circuit during an AF episode shown. Circle indicates the rotor core, also defined by the LAT map showing a region of isochronal crowding and wavefront curvature, leading to slowing in conduction (<0.03 m/s). Red indicates the depolarization phase of the wavefront, and blue, the repolarization phase. The wavefront in the PV propagates around the rotor core, generating rotational activity. (lower panel) Unipolar electrograms (uEGMs) recorded by the 64 channel MEA. The vertical pink line shows the time of recording of the phase map above. Images at 2.5 ms intervals shown. (left) Rotational activity occurrence relative to the total number of beats analysed during 5 s of AF evoked by burst pacing in the presence of $5 \mu\text{g/kg}$ ACh in Sed ($n = 5$) and ExT ($n = 7$) canines. (F) (left) Representative Masson's trichrome staining of $10 \mu\text{m}$ sections of the PV and PV-LA junction from Sed ($n = 6$) and ExT ($n = 5$) preparations. Blue, ECM; purple, myocytes. (F) (right) Fractional area of interstitial ECM deposition in Sed and ExT preparations normalized to the Sed value, computed from Masson's trichrome-stained sections. Four sections from each preparation were analysed. *P*-values computed by Mann-Whitney U test

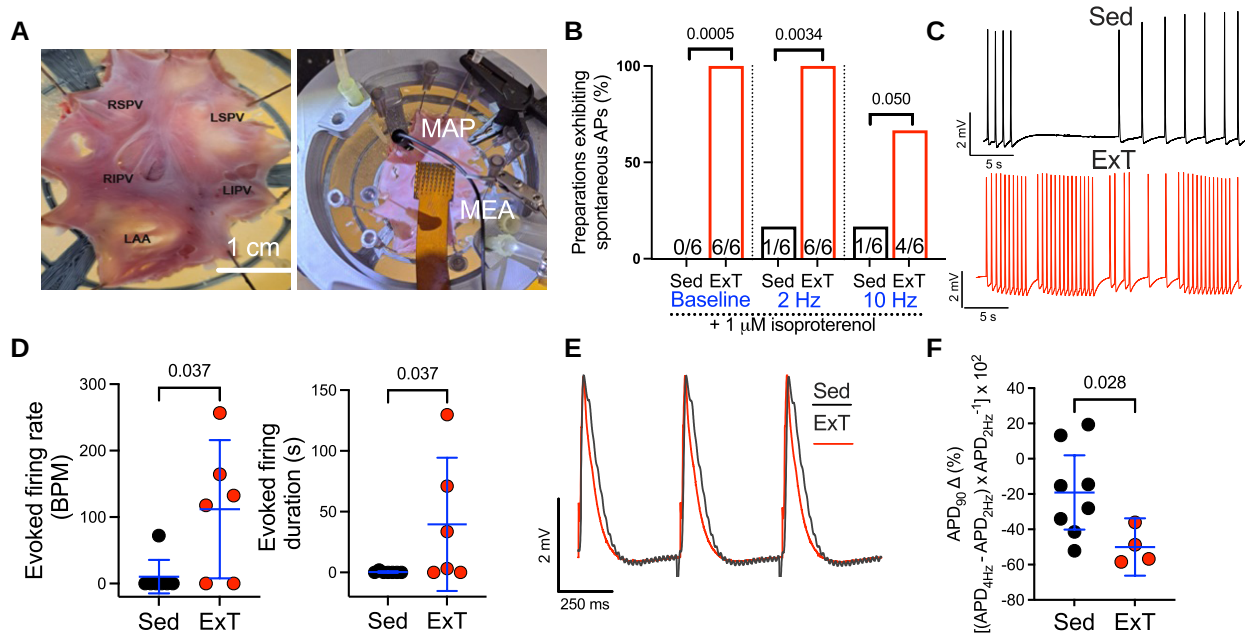


Figure 3 Endurance training enhances intrinsic PV excitability in canines. (A) Canine PV-LA preparations and position of MAP and MEA electrodes in the perfusion chamber. LIPV, left inferior PV; LSPV, left superior PV; RIPV, right inferior PV; RSPV, right superior PV. (B) Summary data showing percentage of PV-LA preparations in which PV ectopic activity could be evoked using 1 μ M isoproterenol or on electrical stimulation for 3 s at 2 and 10 Hz in Sed and ExT canines. Numbers of cases of ectopic activity and preparations analysed per treatment are given in bar chart. (C) Representative MAP recordings of spontaneous PV ectopic activity in isolated PV-LA preparations from Sed and ExT canines following cessation of pacing (2 Hz). (D) Rate (left panel) and duration (right panel) of evoked firing in Sed ($n = 7$) and ExT ($n = 6$) canines following 10 Hz burst pacing in the presence of 1 μ M isoproterenol. (E) Representative MAP recordings in Sed and ExT PV showing shorter APD₉₀ in ExT preparations at baseline. (F) APD₉₀ shortening at 4 Hz relative to a pacing rate at 2 Hz (as a percentage of APD₉₀ at 2 Hz) in the presence of 1 μ M isoproterenol in Sed ($n = 8$) and ExT ($n = 4$) PV. P-values computed by Mann-Whitney U test (D, F) or Fisher's exact test (B)

methods). After 6 weeks, ExT mice showed a 20.5% increase in body mass normalized VO_2 (mL/min/kg–0.75, Figure 4A). Echocardiography confirmed an athlete's-heart phenotype, with increased LV relative wall thickness and larger diastolic LV dimensions (see Supplementary data online, Table S1), together with increased LA anteroposterior diameter (Figure 4B). ExT mice also developed sinus bradycardia and PR prolongation that persisted after combined autonomic blockade (atropine 0.5 mg/kg; propranolol 1 mg/kg), consistent with intrinsic electrophysiological remodelling seen in ExT canines (see Supplementary data online, Table S1 and Supplementary data online, Figure S2).

Inducibility of atrial tachyarrhythmia was assessed in the isolated Langendorff-perfused hearts (an ECG-like electrogram was recorded to monitor electrical activity) using an extrastimulus pacing protocol³⁰ in the presence of the cholinergic agonist carbachol (1 μ M). Examples from an ExT heart are shown in Figure 4C (left panel). Atrial tachyarrhythmia defined as rapid and chaotic electrical activity exceeding 10 s in duration¹⁹ was observed in 0% of Sed and 57.1% of ExT hearts, indicating greater susceptibility in ExT mice (Figure 4C, right panel). Excitability of isolated PV-LA preparations was assessed using MEA mapping of the endocardial surface (Figure 4D, left panel). Electrical stimulation at the right superior PV ostium triggered spontaneous APs on cessation of pacing, the frequency of which

was higher in ExT as compared to Sed preparations (Figure 4D, right panels) indicating increased excitability of ExT PVs, as also observed in canines (Figure 3D).

Compared with nodal tissue and working myocardium, PV myocytes display a distinctive electrophysiological phenotype that may favour triggered activity.³¹ In the PVs, spontaneous AP bursts were frequently seen, and examples recorded with sharp microelectrodes from ExT preparations are shown in Supplementary data online, Figure S3A. Analysis of isochronal activation maps from MEA recordings showed that within a burst, there can be beat-to-beat variability in the direction of conduction from the site of earliest activation (SEA; Supplementary data online, Figure S3A, lower panels). Subtle changes to the location of the SEA ('pacemaker shift') were observed between preparations at baseline and in the presence of 10 μ M noradrenaline (see Supplementary data online, Figure S3B and C). For example, 10 μ M noradrenaline resulted in an inferior shift of the SEA from the right superior PV to the PV-LA junction in 50% of ExT preparations, but only 25% of Sed preparations (see Supplementary data online, Figure S3C). ExT preparations presented with an increased proportion of fractionated electrograms (indicative of conduction inhomogeneity) which corresponded to distinct lines of conduction block in the PV-LA junction (conduction velocity < 0.1 m/s; Figure 4E, left panels). As such, conduction velocity was significantly

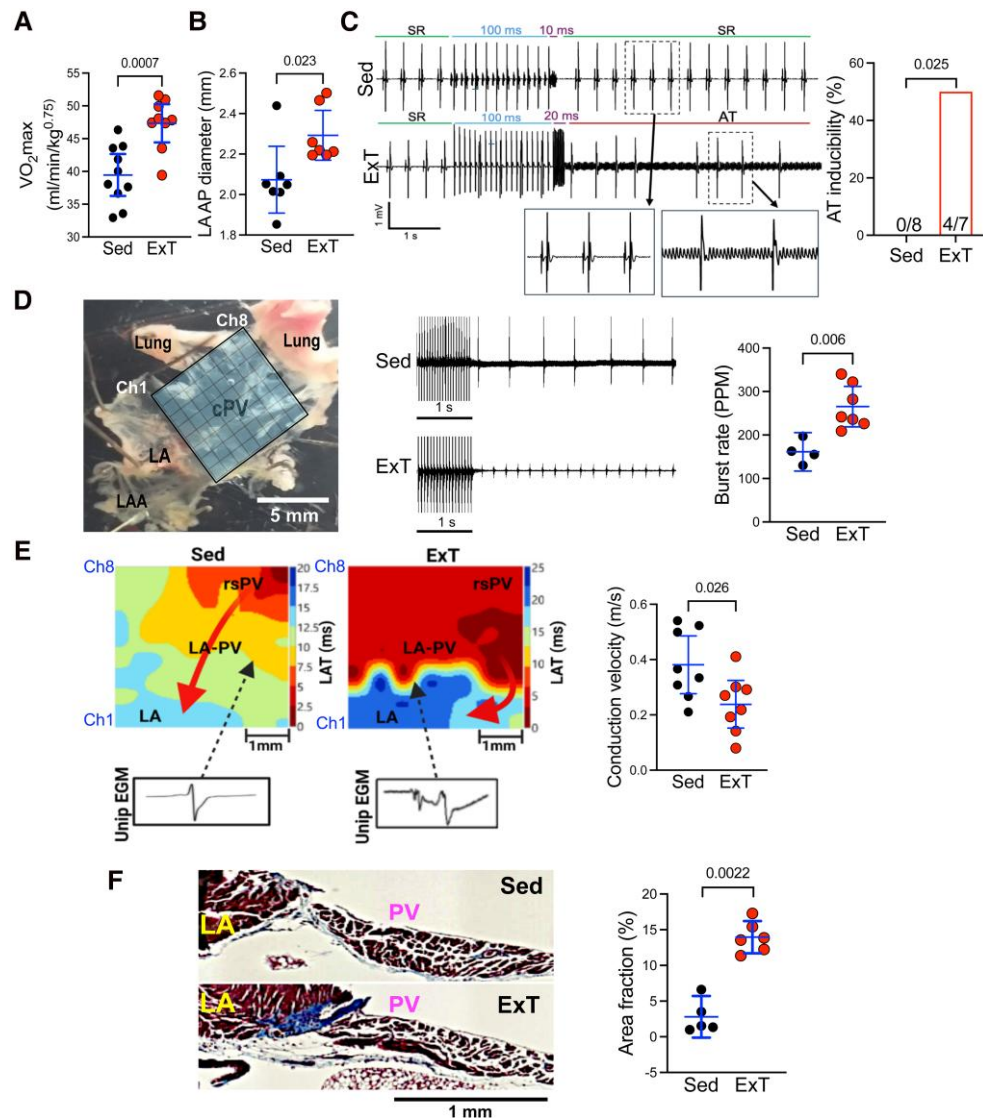


Figure 4 Athlete's heart phenotype, enhanced AF susceptibility, and PV-LA junction remodelling in ExT mice. (A) Normalized $\text{VO}_{2\text{max}}$ of Sed ($n = 8$) and ExT ($n = 8$) mice following 6 weeks of intensity-controlled interval treadmill training. (B) Left atrial anteroposterior (LAAP) diameter in Sed ($n = 8$) and ExT ($n = 7$) mice. (C) (left) Representative ECG traces from Langendorff-perfused Sed and endurance-trained ExT mouse hearts during baseline sinus rhythm (SR) and during programmed electrical stimulation under $1 \mu\text{M}$ carbachol. All hearts received the same protocol: 20 S1 stimuli at 100-ms cycle length followed by S2–S10 decremental miniburst pacing with coupling intervals stepped from 58 ms down to 8 ms (as labelled). In the Sed heart, SR is maintained even at a 10-ms coupling interval, whereas in the ExT heart, the 20-ms train already provokes sustained atrial tachyarrhythmia (AT) sustained for at least 10 s. Dashed boxes indicate segments shown at higher magnification (insets). (C) (right) Summary of susceptibility to pacing-induced atrial tachyarrhythmia in Sed vs. ExT hearts (percentage of hearts with AT). Numbers of AT events and total hearts analysed are shown on the bar chart. (D) (left) Mouse PV-LA preparation showing MEA position. Channels 1 and 8 (Ch1 and Ch8) indicate the orientation of the MEA in this and all representative maps. cPV, common PV. (D) (middle) Representative unipolar electrograms from Sed and ExT preparations during 10 Hz pacing (for 3 s) from the RSPV (pacing-induced beats during final 1 s of pacing shown) and following the cessation of pacing (spontaneous beats shown). (D) (right) Summary data showing burst rate following pacing in Sed ($n = 4$) and ExT ($n = 7$) PV. PPM, pulses per minute. (E) Representative MEA isochronal maps of LAT during an ectopic beat arising in the PV in Sed and ExT preparations. rsPV, right superior PV. Representative unipolar electrograms (UnipEGMs) at the locations indicated are given in the insets. Typical biphasic unipolar electrograms were observed at the PV-LA junction in Sed PV, while in ExT PV unipolar electrograms were often fractionated or displayed double peaks. Arrows indicate wavefront propagation path. (E) (right) Summary data of conduction velocity in Sed ($n = 8$) and ExT ($n = 8$) preparations. (F) (left) Representative Masson's trichrome staining of $10 \mu\text{m}$ sections of the PV-LA junction from Sed and ExT preparations. Blue, ECM; red, myocytes. (F) (right) Fractional area of interstitial ECM deposition in Sed ($n = 6$) and ExT ($n = 7$) preparations computed from Masson's trichrome-stained sections. Four sections from each preparation were analysed. *P*-values computed by Student's *t* test (panels A–B, E), Mann–Whitney *U* test (D), or Fisher's exact test (C).

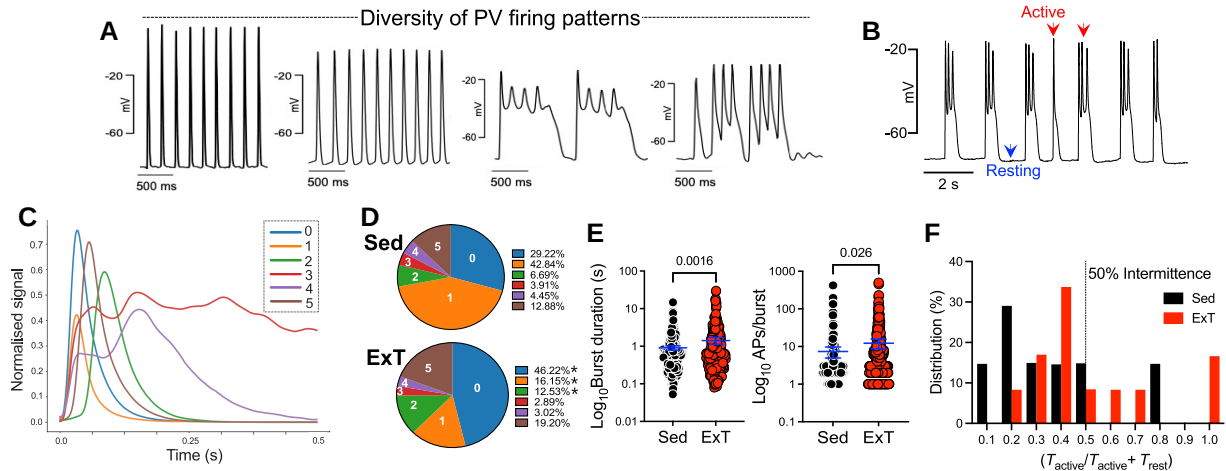


Figure 5 PV AP characteristics and excitability are modified by endurance exercise. (A) Representative intracellular APs recorded using sharp microelectrodes in mouse Sed PV preparations demonstrating exemplar AP subtypes in the PV. (B) Representative intracellular AP recording from a mouse Sed PV preparation demonstrating the typical burst firing pattern. (C) Normalized AP waveform templates for the six PV AP subtypes. (D) Pie charts showing proportion of six detected AP subtypes computed as the fraction of events of a given shape in Sed (top panel) and ExT (bottom panel) preparations. Asterisks denote FDR-corrected $P < .001$ from binary logistic regression at the single-AP level (group as predictor; SEs clustered by animal; FDR-corrected across shapes 0–2). (E) Burst duration (left) and number of APs per burst (right) in Sed and ExT PV myocytes; each point represents a single burst. P -values derived from Welch's t tests on log10-transformed data. Points represent individual observations; horizontal lines indicate the group mean; error bars indicate 95% confidence intervals. (F) Histogram showing distribution of intermittence in Sed and ExT PV. Values >0.5 indicate sustained burst activity. Value distribution as median, 25% and 75% percentile: Sed (0.27, 0.20, 0.46); ExT (0.43, 0.35, 0.67)

reduced in ExT PVs as compared to Sed PVs (Figure 4E, right panel). Akin to canines, Masson's trichrome staining revealed increased ECM deposition in ExT preparations that was particularly pronounced at the interface between the PV and the LA, which coincides with conduction slowing and block in this region (Figure 4F; see Supplementary data online, Figure S4 for further detail). Next, intracellular APs were recorded with sharp microelectrodes from endocardial PV regions of intact PV–LA preparations from Sed ($n = 12$) and ExT ($n = 9$) mice. Pulmonary vein electrical activity was highly heterogeneous in both groups, with atrial-like APs, pacemaker-like APs with diastolic depolarization, and APs with early afterdepolarizations and burst firing patterns (Figure 5A and B; Supplementary data online, Figure S3A). In contrast to previous work,³¹ specific AP morphologies were not restricted to specific regions within the PV. To quantify this heterogeneity objectively, we designed pattern-recognition software to classify AP morphologies and burst firing (see Supplementary Methods). This identified six reproducible AP types (Figure 5C) across preparations and showed a greater proportion of pacemaker-like APs in ExT PVs (Sed, 6.7%; ExT, 12.5%; shown in green in Figure 5D; Supplementary data online, Figure S5). Burst activity in ExT preparations was also more sustained, with longer burst duration and more APs per burst (Figure 5E; Supplementary data online, Figure S6). Intermittence, defined as the burst duration (T_{active}) expressed as a percentage of the sum of the burst and rest durations ($T_{\text{active}} + T_{\text{rest}}$; Figure 5F), demonstrated that the time spent during the burst (i.e. the active phase) was increased in ExT as compared to Sed preparations. In ExT preparations, the proportion of bursts with durations longer than 1.01 s and 4.01 s were

greater than in Sed preparations; correspondingly, the proportion of shorter bursts was less in ExT preparations than in Sed preparations (see Supplementary data online, Figure S6).

Molecular signature of training-induced PV remodelling

To define the transcriptional programme associated with ExT-induced PV remodelling, we performed bulk RNA-seq on biopsies of the mouse common PV (Sed $n = 8$; ExT $n = 7$). Of 55 401 transcripts measured, a total of 3590 transcripts met the prespecified threshold for differential expression between groups ($P < .05$, FDR < 0.1 ; Supplementary data online, Figure S7). Among the most prominent upregulated ion channel transcripts was *Hcn4* (see Supplementary data online, Figure S7A and B), consistent with the increased occurrence of pacemaker-like AP behaviour observed in ExT PV (Figure 5D). Pathway enrichment analysis highlighted activation of immune-inflammatory and profibrotic programmes in ExT PVs, including TGF β - and NF κ B-related signalling, IL6/IL11, CXCL chemokines (e.g. Cxcl1/2/3/5/11), and cytokine receptors (e.g. Tnfr1) (see Supplementary data online, Figure S7). To account for mixed cell populations in bulk RNA-seq, we used digital cytometry (CIBERSORTx) with published murine PV single-cell reference data to estimate cell-type composition (see Supplementary data online, Table S3). This suggested changes in the proportion of different cell types in the PV following ExT: a significant increase in the proportion of one fibroblast subtype, a significant decrease in the proportion of another fibroblast subtype, and an increase (trend only) in the proportion

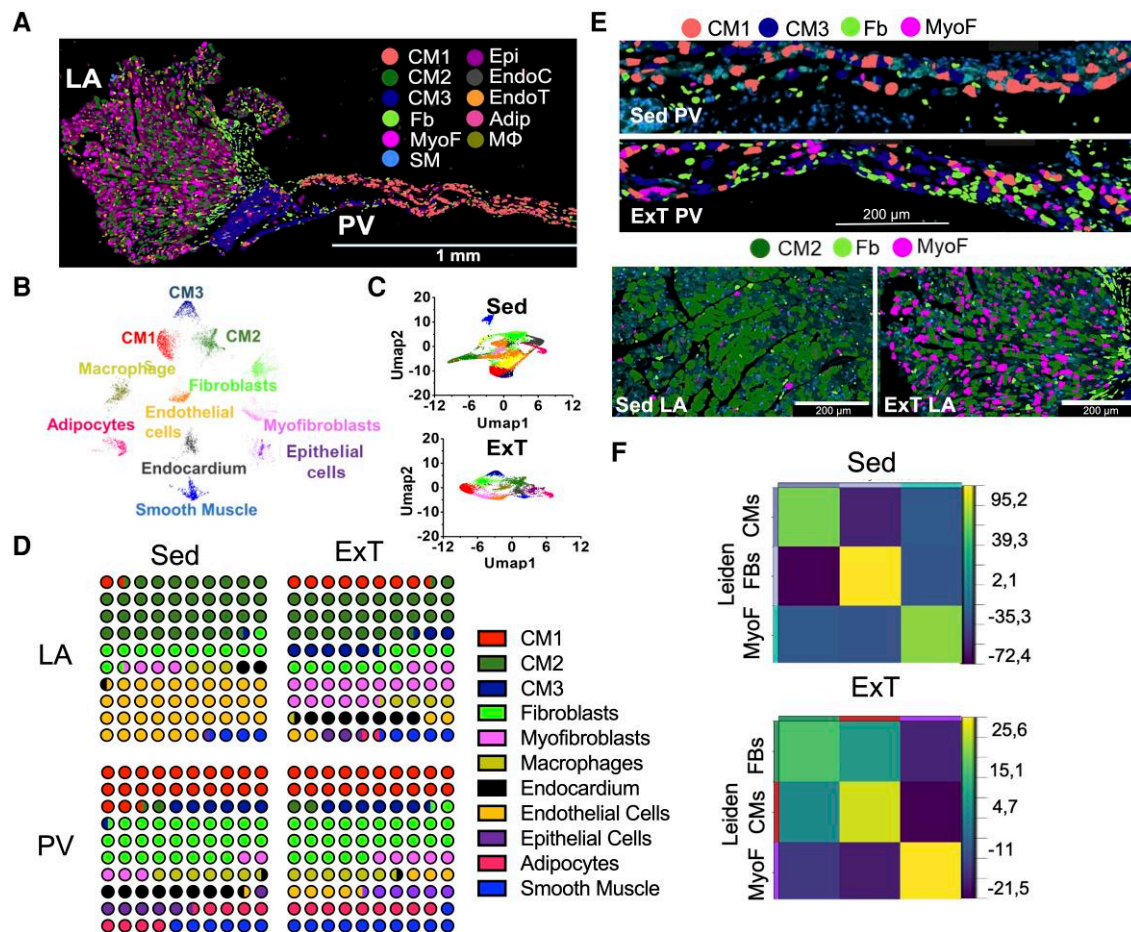


Figure 6 Molecular landscape of the PV-LA junction of the mouse at subcellular spatial resolution. (A) Representative 5 µm section of ExT mouse PV-LA junction in Xenium spatial *in situ* transcriptional study indicating heterogeneity of cell types detected in the PV-LA junction. Each colour-labelled dot corresponds to a specific transcript captured from a particular location in the tissue section. Eleven cell types were identified across six preparations and were assigned to a unique colour (CM1, red; CM2, dark green; CM3, dark blue; macrophage, dark yellow; fibroblasts, green; adipocytes, pink; endothelial cells, orange; myofibroblasts, magenta; endocardium, dark grey; epithelial cells, purple; smooth muscle, blue). (B) Uniform manifold approximation and projection (UMAP) representation of cell types identified across Sed ($n = 3$) and ExT ($n = 3$) PV preparations. (C) UMAP in Sed samples (Top) and ExT (bottom). (D) Waffle plots demonstrating mean cell fractions of a specific cell type (colour coded, key shown in figure) in LA and PV regions of Sed and ExT preparations. (E) (top) Representative image of fibroblasts (green) and myofibroblasts (magenta) showing spatial distribution across PV regions in Sed and ExT mice. PV-specific CMs highlighted. (E) (lower) Representative image of fibroblasts (green) and myofibroblasts (magenta) showing spatial distribution across LA of Sed and ExT mice. LA-specific CM population (CM2) highlighted. (F) Heatmaps depicting Z-scores from neighbourhood enrichment analysis, indicating spatial interactions between cell-type pairs in Sed (top) and ExT (bottom) conditions. Significant interactions ($|Z| > 1.96$, $P < .05$) show enrichment or depletion, with nonsignificant interactions ($|Z| \leq 1.96$, $P > .05$) reflecting random spatial patterns

of one type of cardiomyocyte (see [Supplementary data online, Figure S7C](#)).

These findings, together with the marked electrophysiological and histological phenotype described above, motivated spatially resolved single-cell analyses of the PV-LA junction. We applied subcellular-resolution targeted *in situ* transcriptomics (10x Genomics Xenium) to FFPE sections of the mouse PV-LA junction ([Figure 6A](#)), using a 450-gene panel enriched for ion channel, inflammatory and ECM transcripts relevant to AF informed by our bulk RNAseq data (see [Supplementary data online, Tables S4 and S5](#); see [Supplementary Methods](#)). After quality control, 96 879 cells from three Sed and three ExT preparations were

retained for analysis. Unsupervised clustering resolved major cell populations (cardiomyocytes, fibroblasts, myofibroblasts, endothelial and smooth muscle cells, macrophages, and others), enabling direct comparison of regional composition and spatial organization ([Figure 6B and C](#)). At the level of major cell classes, macrophage abundance was not modified by region or training status, while other immune populations were detected inconsistently across sections. In contrast, fibroblast and myofibroblast populations showed clear training- and region-associated differences: fibroblasts were enriched in ExT PV (Sed: 17.1%; ExT: 27.4%; $P = .023$, χ^2 test; [Figure 6D](#)), and myofibroblasts were increased in ExT LA (Sed: 3.9%; ExT: 18.7%; $P = .001$; χ^2

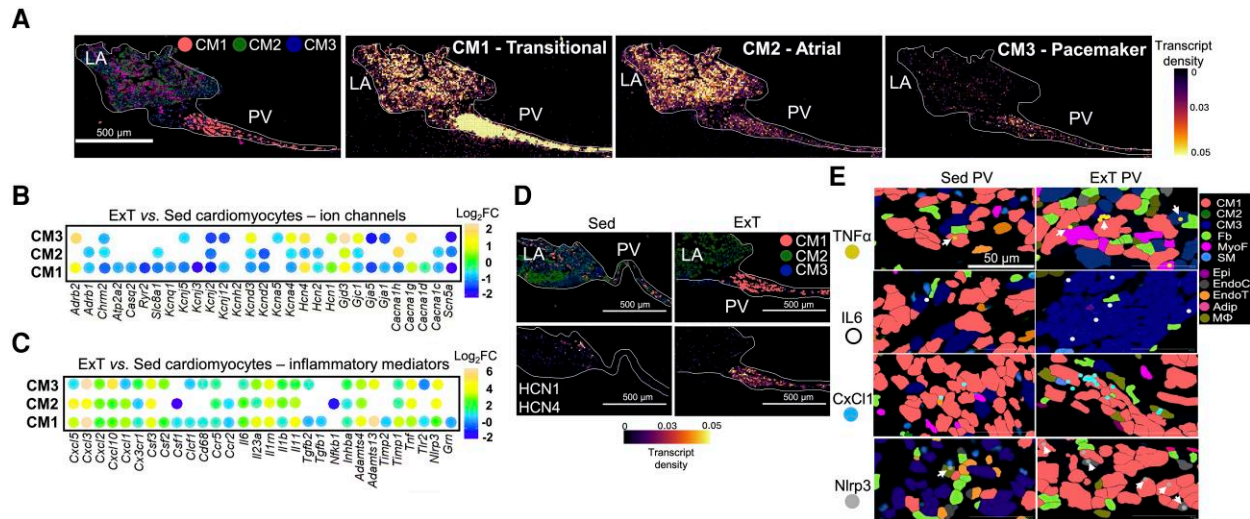


Figure 7 Ion channel remodelling and pro-inflammatory signalling in ExT PV myocytes. (A) Xenium image of PV–LA junction (left panel) and transcript density plots for CM markers giving density of marker genes in tissue per bin ($10 \times 10 \mu\text{m}$ in size). Colour gradient in legend indicates the number of corresponding transcripts captured in each bin. Highest transcript density (0.05) given in yellow and lowest (0) in purple. (B–C) Dot plot of transcripts encoding key ion channels (top) and proteins related to inflammation (bottom) showing significant (Mann–Whitney U test, FDR < 0.05) dysregulation between ExT and Sed groups in Xenium experiments. Colour legend describes upregulated transcripts with (green–yellow–beige) and downregulated transcripts with (green–cyan–blue). Fold changes given in logarithmic scale. (D) Representative spatial distribution of myocyte subtypes in PV–LA junction (top) and corresponding *Hcn* channel transcript density plots (bottom). Colour gradient in legend indicates the number of corresponding transcripts captured in each bin. Highest transcript density (0.05) given in yellow and lowest (0) in purple. (E) Representative spatial distribution of selected pro-inflammatory transcripts in PV cell types of Sed and ExT mice: TNF α (yellow), IL-6 (white), Cxcl1 (cyan), and NLRP3 (grey). Cell types are demarcated by colour coding and key provided in figure

test; Figure 6D), consistent with the ECM expansion quantified histologically (Figure 4F). Neighbourhood analysis further indicated altered microanatomical organization in ExT tissue, including enrichment of fibroblast–myocyte proximity (e.g. FB2–CM1; Figure 6F), supporting increased local fibroblast–myocyte interactions at the PV–LA interface.

Given the electrophysiological phenotype (Figures 4 and 5), we next examined cardiomyocyte heterogeneity and remodelling. Myocytes segregated into three reproducible clusters (CM1–CM3; Figure 7A and B; Supplementary data online, Table S5; Supplementary data online, Figure S8) with distinct ion channel signatures: CM3 showed a pacemaker-like profile (high *Hcn1*, *Hcn4*, *Cacna1d*, *Cacna1g*), CM2 was atrial-like (*Cacna1c* enriched), and CM1 exhibited an intermediate/transitional phenotype. Pacemaker-like CM3 myocytes were enriched in the PV sleeves, atrial-like CM2 myocytes were enriched in the LA myocardium, and transitional CM1 myocytes spanned the junction. Training altered expression of key ion channel transcripts (Figure 7B and D; Supplementary data online, Figures S9–S11). Exercise training PV samples showed increased expression of the pacemaker programme (*Hcn1*, *Hcn4*, *Cacna1d*, *Cacna1g*), consistent with increased PV spontaneous activity/excitability (Figure 4D) and increased frequency of pacemaker-like APs (Figure 5D) and with bulk RNA-seq showing *Hcn4* upregulation (see Supplementary data online, Figure S7A and B). Additionally, reduced *Scn5a* and *Gja1* across myocyte types (consistent with slowed conduction) and decreased *Cacna1c* in CM1/CM2 were observed (consistent with atrial remodelling

and action potential duration shortening; Supplementary data online, Figures S5 and S6). Other training-induced transcript changes to ion channels and Ca^{2+} handling proteins are considered further in Supplementary data online, Table S5 and accompanying Supplementary Discussion. Strikingly, there was a robust upregulation of inflammatory genes within PV myocytes (Figure 7C and E), including *Nlrp3*, NF κ B pathway components, cytokines/chemokines (*Tnf*, *Il6*, *Il1b*, *Il11*, *Cxcl3/5/19*, *Ccr2/5*), and fibrosis-related regulators (*Tgfb1/2*, *Timp1/2*). Selected transcript-level changes were corroborated at the protein level by immunolabelling, which confirmed increased expression of HCN1, HCN4, $\text{Ca}_v1.3$, TNF α , and immune cell expansion labelled with the pan-leukocyte marker CD45 (see Supplementary data online, Figures S10 and S11). Together, these findings localize ion channel remodelling and pro-inflammatory signalling to the PV–LA myocyte compartment, linking the molecular profile to the electrophysiological changes and fibro-inflammatory substrate shown in Figures 4–6. This is consistent with prior studies linking pro-inflammatory signalling in myocytes with structural and electrical remodelling in AF.^{14,15,32–37}

Exercise training-induced PV–LA junction remodelling is proarrhythmogenic

We next used biophysically detailed computer models of human atrial²⁴ and PV myocytes²⁵ to test whether the remodelling observed experimentally is sufficient to reproduce key arrhythmogenic features in a human electrophysiological context. We first

asked whether the PV transcriptomic changes have the potential to increase PV automaticity. The Dorn *et al.*²⁵ PV myocyte model was used to represent a transitional (CM1) phenotype and adapted to model pacemaker-like (CM3) myocytes based on ion channel transcript differences (see [Supplementary data online, Table S6](#)). Since both CM1 and CM3 myocytes express HCN transcripts and I_f has been recorded in PV myocytes, I_f was included in both models. The conductance of I_f (g_f) in PV myocytes is not known, and in this study, a range of g_f values from 0 to 0.05 nS/pF were used. In the study of Takagi *et al.*,³⁸ the density of I_f was about -1.9 pS/pF at -140 mV, and this corresponds to a conductance between 0.01 and 0.03 nS/pF (see [Supplementary data online, Figure S12](#)). [Figure 8A](#) shows fast time base recordings of steady-state APs, and [Figure 8B](#) shows APD₉₀ and the maximum diastolic potential (MDP) in the three myocyte types in the Sed and ExT conditions—at various levels of g_f in the case of the transitional (CM1) and pacemaker-like (CM3) myocytes. Atrial-like (CM2) myocytes exhibited stable diastolic potentials (~ -80 mV) and long APD, closely matching experimental recordings ([Figure 5](#), [Supplementary data online, Figure S6](#)). Transitional (CM1) and pacemaker-like (CM3) myocytes showed more positive diastolic potentials and diastolic depolarization when g_f conductance was introduced. The pacemaker-like (CM3) myocyte is predicted to show spontaneous activity in the Sed condition, and, furthermore, the spontaneous activity is predicted to be faster and seen over a wider range of g_f in the ExT condition ([Figure 8C and D](#)), whereas the atrial-like (CM2) and transitional (CM1) myocytes are predicted not to show spontaneous activity in either condition ([Figure 8C and D](#)). These predictions are consistent with experimental findings which show that ExT increases PV ectopic activity ([Figure 5](#); [Supplementary data online, Figure S6](#)). Together, the simulations indicate that exercise-induced ion channel remodelling enhances ectopic pacemaker activity in PV myocytes.

The second question to be addressed with computer modelling is whether the structural and transcriptomic remodelling after ExT has the potential to make the atrial muscle more likely to sustain AF once initiated. A mesh of the LA was generated from cine-magnetic resonance images of a healthy 64-year-old male ([Figure 8E](#)), and atrial and PV myocytes were again modelled by the Courtemanche *et al.*²⁴ and Dorn *et al.*²⁵ models (full detail given in [Supplementary Methods](#)). To simulate the effects of ExT, the LA mesh was enlarged by 30% to account for the LA enlargement,^{21,39} the fibrotic region at the PV–LA antrum was increased by 14% ([Figure 4F](#)), ionic conductances in the PV and atrial myocytes were scaled based on the changes in ion channel transcript expression (see [Supplementary data online, Table S7](#)), and the diffusion coefficient was reduced to 68% to account for the decrease in gap junction expression (see [Supplementary data online, Table S7](#)). Arrhythmia vulnerability of the LA was assessed by applying the PEERP (Pacing at the End of the Effective Refractory Period) protocol⁴⁰ at pacing sites on the superior and inferior faces of each of the four PVs. A pacing site was defined as inducible if an arrhythmia was sustained for at least 1.5 s after the end of the pacing protocol. In the Sed condition, re-entry could not be initiated at any site. However, in the ExT condition, re-entry was induced at four of the eight PV pacing sites: superior and inferior faces of the left superior PV, superior face of the right superior PV,

and the inferior face of the left inferior PV. An example of pacing-induced re-entry at one of the sites (superior face of the LSPV) in the ExT condition is shown in [Figure 8E](#), and a movie of pacing-induced re-entrant rotational activity is shown in the [Supplementary Material](#). In conclusion, these *in silico* studies suggest that the measured training-induced transcriptomic and structural remodelling changes can initiate and maintain AF in a human atrial context.

Discussion

Summary

In this study, we interrogated exercise-induced PV and PV–LA junction remodelling across complementary canine and murine endurance-training models. Across species, training increased PV excitability and remodelled the PV–LA junction, with conduction slowing and heterogeneity that co-localized with ECM expansion. In mice, sharp microelectrode recordings revealed heterogeneous PV action potentials with a shift towards pacemaker-like firing and prolonged burst activity. These functional changes were consistent with spatial transcriptomic resolution of increased pacemaking ion channels in PV myocytes and a myocyte-intrinsic inflammatory/profibrotic programme at the PV–LA region. Human-scale simulations implementing the experimentally measured remodelling increased PV pacemaker propensity and enabled sustained re-entry in an athlete-scale LA compared with baseline, supporting the plausibility that PV/PV–LA remodelling of the type quantified here can generate clinically relevant arrhythmogenic behaviour when embedded in human atrial geometry.

The PVs are a dominant trigger source in clinical AF, yet relatively few studies have examined, in depth or in translatable models, how latent PV arrhythmogenic activity becomes manifest and degenerates into atrial arrhythmogenesis. While our mechanistic emphasis is on PV sleeves and the PV–LA region, training-associated remodelling is not confined to this interface; accordingly, we also analysed atrial myocytes in the spatial datasets and incorporated atrial cell remodelling in the human-scale simulations. Three mechanistic aspects merit further consideration: First, endurance training increases PV excitability and automaticity; the expansion of pacemaker-like PV myocyte behaviour (and associated transcriptomic signatures) provides a plausible basis for heightened PV trigger propensity, particularly under physiological stress. Notably, although AF in athletes is often attributed to heightened vagal tone, sequential autonomic blockade in our canine cohort did not provide evidence of increased parasympathetic or sympathetic tone, while intrinsic heart rate was reduced, supporting predominantly intrinsic electrical remodelling rather than altered resting autonomic tone in this model. This observation complements chamber-specific HCN remodelling: while I_f remodelling in the sinus and AV node explains training-related bradycardia and AV conduction slowing,^{22,23,27} pulmonary vein sleeves show a reciprocal pacemaker-like programme that is pro-ectopic. Similarly, onset and maintenance of age-related⁴¹ or tachypacing-induced AF in dogs⁴² have been linked to lower HCN channel expression in the sinoatrial node coupled with higher expression in the PV and LA. Further investigation of the epigenetic and transcriptional programmes that dictate region-specific expression of HCNs and other pacemaking-related channels may reveal

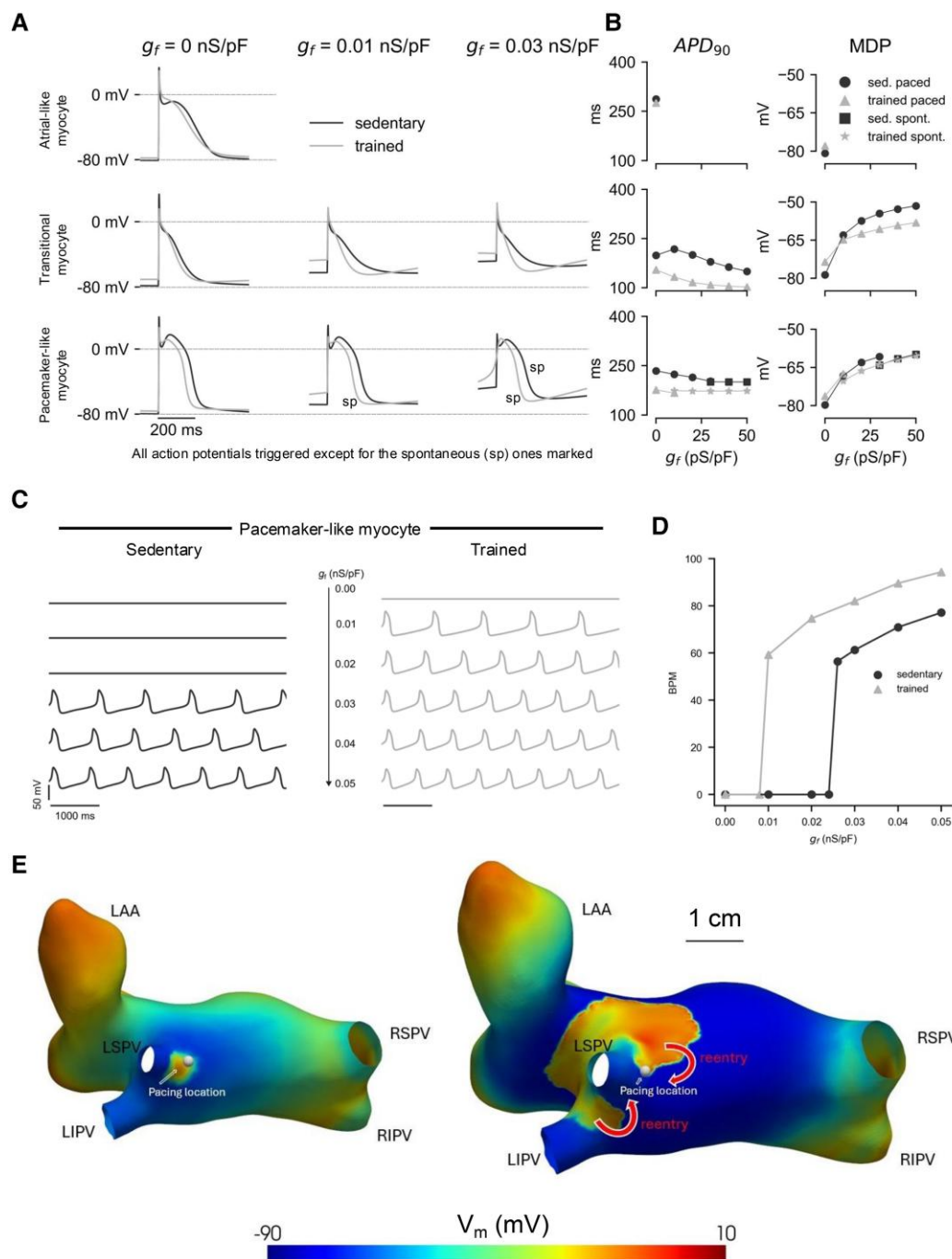


Figure 8 Exercise training-induced remodelling is proarrhythmic. (A) Simulated heterogeneous AP morphology. Steady-state APs for the three myocyte model types (atrial-like, transitional, pacemaker-like) in Sed (black traces) and ExT (grey traces) conditions are shown at three values of g_r . When applied pacing at 60 BPM overdrives spontaneous activity, the paced AP is shown; when spontaneous activity overdrives applied pacing, the spontaneous AP (sp) in the absence of applied pacing is shown. (B) Relationship between APD_{90} and the MDP for the three myocyte model types in Sed (black traces) and ExT (grey traces) conditions and g_r . (C) Spontaneous APs in the pacemaker-like myocyte model in the Sed and ExT conditions at various values of g_r . Flat lines indicate the absence of spontaneous activity. (D) Exercise training increases spontaneous activity in the pacemaker-like myocyte model. The relationship between the rate of spontaneous APs and g_r in the pacemaker-like myocyte model in the Sed and ExT conditions is shown. In (A–D), g_r is a variable, because its value is not known and measurement of spontaneous APs were made in the absence of pacing. (E) Simulation of the effects of exercise training. Meshes of the left atrium of a healthy 64-year-old male, showing PVs (green), PV–LA junction (yellow), and fibrosis at the PV–LA junction (red) overlaid on the atrial surface (blue), are shown. The original mesh was assumed to correspond to the Sed condition and is shown on the left, and the modified mesh on the right was taken as the ExT condition (see text for a description of the changes made). The superior face of the LSPV was paced, and the colour-coded membrane potential (see scale) at the end of pacing is shown. Only pacing in the ExT case resulted in reentry

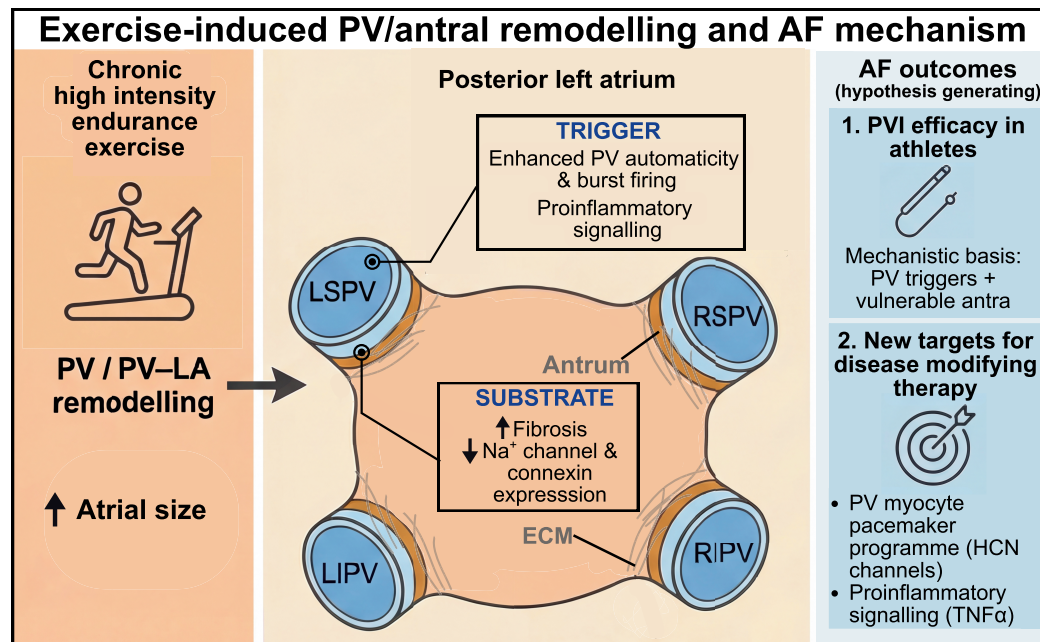


Figure 9 Central illustration linking exercise-induced remodelling to clinical AF outcomes. Illustration summarizing how endurance training remodels the PV sleeves and PV–LA junction to increase the likelihood that PV activity initiates sustained AF and highlights the measured mechanistic features that inform PV-targeted therapy and upstream mechanistic candidates

mechanistic entry points for suppressing PV trigger activity while preserving physiological nodal function.

Second, it is likely that specific electrophysiological characteristics of the PV–LA junction predispose to AF initiation, because ectopy from other regions of the atrium such as the crista terminalis does not commonly degenerate into AF, and in some people rapid sustained atrial tachycardia from the PVs can propagate to the LA without initiating AF,⁴³ implying that a permissive PV–LA substrate is often required. Our data support a coupled mechanism in endurance training: (i) PV myocyte ion channel remodelling increases trigger propensity while (ii) PV–LA ion channel remodelling and fibroblast-associated ECM expansion promote conduction delay and heterogeneity (reduced *Scn5a* and *Gja1* are expected to slow conduction *via* a decrease in I_{Na} and intercellular coupling,^{44,45} and reduced *Kcnj2* is expected to depolarize myocytes and further decrease I_{Na} *via* inactivation, compounding conduction slowing).

Third, while inflammatory signalling has been mechanistically linked to atrial electrical remodelling,⁴⁶ this study demonstrates a PV myocyte-intrinsic inflammatory/profibrotic programme. Among the pathways identified, $TNF\alpha$, a mechanosensitive cytokine with recognized electrophysiological and profibrotic effects, is of particular interest. $TNF\alpha$ elevations have been reported after endurance competition⁴⁷ and correlate with post-exercise cardiac dysfunction.⁴⁸ In mice, $TNF\alpha$ inhibition prevents exercise-induced atrial remodelling and AF susceptibility,^{14,15} and $TNF\alpha$ induction in HCN4-expressing cells heightens AF inducibility.⁴⁹ Defining the causal sequence linking pro-inflammatory signalling with PV excitability and PV–LA substrate formation may help distinguish adaptive from maladaptive responses to high training loads and for identifying upstream interventions.

Limitations

Although the use of complementary species and platforms strengthens inference, residual confounding related to species, preparation, and protocol-specific differences cannot be fully excluded. Our study focused deliberately on the PV and PV–LA junction; however, atrial remodelling in AF may extend beyond this region, including more diffuse left- and right-atrial changes that were not comprehensively assessed here. Our findings cannot be extrapolated directly to human athlete AF, where remodelling likely accrues over decades. Nonetheless, by studying 4 months of high-intensity endurance training (approximately five human years) in a canine model with close anatomic, haemodynamic, and electrophysiological parallels to the human heart, we provide a foundational large-animal step in defining exercise-linked AF mechanisms. Spatial ion channel findings are predominantly at the mRNA level (with protein-level validation for selected targets), and definitive subtype-resolved ionic current measurements will require new PV-specific transgenic tools and prospective isolation strategies. Finally, we did not vary training dose or include a training-adaptation (detraining) arm, so we cannot comment on reversibility of PV/PV–LA remodelling or whether exercise modification reduces AF burden; these questions are being addressed in randomized training-adaptation trials in endurance athletes with paroxysmal AF (e.g. NCT04991337/NEXAF).

Clinical implications (see Figure 9)

- (i) **Mechanistic explanation for PVI success in athletes.** Across complementary approaches (*in vivo/ex vivo* electrophysiology, cellular phenotyping, and human-scale simulations),

endurance training increased PV excitability and automaticity and produced PV–LA junction remodelling that was consistent with conduction slowing and greater susceptibility to re-entry. Together, these findings provide a mechanistic basis for why antral PV isolation, by electrically excluding PV sleeves and adjacent peri-antral tissue, can be clinically effective in endurance-trained individuals with AF.²⁰ We do not propose an athlete-specific, uniquely ablation-responsive PV lesion; rather, endurance training amplifies canonical PV and peri-antral mechanisms implicated in AF initiation and maintenance.

- (ii) **Risk stratification:** Identifying endurance athletes at highest AF risk remains an unmet need. Although PV triggers commonly initiate AF, 24-h monitoring studies in athletes have not consistently shown a higher burden of premature atrial beats than in sedentary controls.⁵⁰ Routine monitoring typically does not identify ectopy origin, and therefore, global ectopy counts are likely an incomplete proxy for PV involvement. Our data motivate the hypothesis that PV–LA/antral substrate measurements such as peri-antral fibrosis distribution on atrial imaging and invasive peri-antral substrate mapping in ablation cohorts may associate with AF susceptibility beyond ectopy burden alone.
- (iii) **Potential pharmacologic targets for AF prevention.** Intervention testing was outside the scope of this study, but our spatially resolved molecular data identify PV-enriched pathways for mechanistic validation and future upstream interventional work aimed at modifying PV triggers and PV–LA substrate formation. These include pacemaker-linked ion channel programmes (including HCN/I_f and Ca_v1.3-associated signatures) and TNF α -linked inflammatory/profibrotic signalling. The procedural accessibility of the PV–LA antrum raises the possibility of local PV-targeted modulation strategies; however, feasibility, safety, and efficacy will require dedicated pre-clinical and clinical testing.
- (iv) **Relevance to AF pathophysiology beyond athletes.** Although endurance training was the perturbation used here, the two components examined likely recur in many AF settings: PV-triggered initiation and variable susceptibility of the peri-antral region to conduction slowing, heterogeneity, and fibrosis. Our data provide a rationale to test, in other AF models and disease settings, whether such remodelling contributes to AF initiation and maintenance, alongside established global atrial cardiomyopathy measures.

Supplementary data

Supplementary data are available at [European Heart Journal](#) online.

Declarations

Disclosure of Interest

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Data Availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Ethical Approval

Experiments in canines were approved by the Ethical Committee for the Protection of Animals in Research at the University of Szeged, Hungary (approval numbers: I-74-15-2017, I-74-24-2017), and the Ministry of Agriculture and Rural Development (XIII/509/2022), in accordance with Directive 2010/63/EU. Murine studies were approved by the University of Manchester and Imperial College London, complying with the UK Animals (Scientific Procedures) Act 1986.

Pre-registered Clinical Trial Number

Not applicable.

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