CARDIAC MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL CHANGES INDUCED BY SUSTAINED, HIGH-INTENSITY ENDURANCE TRAINING IN LARGE ANIMAL EXPERIMENTAL MODELS

Ph.D. thesis

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LIST OF PUBLICATIONS

The publications related to the subject of the Ph.D. thesis:

I.) **A. Polyák,** L. Topal, N. Zombori-Tóth, N. Tóth, J. Prorok, Zs. Kohajda, Sz. Déri, V. Demeter-Haludka, P. Hegyi, V. Venglovecz, G. Ágoston, Z. Husti, P. Gazdag, J. Szlovák, T. Árpádffy-Lovas, M. Naveed, A. Sarusi, N. Jost, L. Virág, N. Nagy, I. Baczkó, A. S. Farkas, A. Varró. Cardiac electrophysiological remodelling associated with enhanced arrhythmia susceptibility in a canine model of elite exercise.

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II.) Péter Kui*, **Alexandra Polyák*,** Nikolett Morvay, László Tiszlavicz, Norbert Nagy, Balázs Ördög, Hedvig Takács, István Leprán, András Farkas, Julius Gy. Papp, Norbert Jost, András Varró, István Baczkó and Attila S. Farkas. Long-term endurance exercise training alters repolarization in a new rabbit athlete's heart model.

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VI.) A.O. Verkerk, I.J. Doszpod, I. Mengarelli, T. Magyar, **A. Polyák,** B. Pászti, I.R. Efimov, R. Wilders, and I. Koncz. *Acetylcholine Reduces L-Type Calcium Current without Major Changes in Repolarization of Canine and Human Purkinje and Ventricular Tissue.* BIOMEDICINES 10: 11 Paper: 2987, 20 p. (2022) doi: doi.org/10.3390/biomedicines10112987. (**IF:** 5.612, **Q2**)

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VIII.) P. Gazdag, K. Oravecz, K. Acsai, V. Demeter-Haludka, B. Ördög, J. Szlovák, Zs. Kohajda, **A. Polyák,** B.A. Barta, A. Oláh, T. Radovits, B. Merkely, J.Gy. Papp, I. Baczkó, A. Varró, N. Nagy, and J. Prorok. *Increased $Ca2^+$ content of the sarcoplasmic reticulum provides arrhythmogenic trigger source in swimming-induced rat athlete's heart model.* SCIENTIFIC REPORTS 10: 1 Paper: 19596, 13 p. (2020), doi: https://doi.org/10.1038/s41598-020-76496-2. (IF: 4.379, **D1**)

IX.) P. Orvos, B. Pászti, L. Topal, P. Gazdag, J. Prorok, **A. Polyák,** T. Kiss, E. Tóth-Molnár, B. Csupor-Löffler, Á. Bajtel, A. Varró, J. Hohmann, L. Virág, and D. Csupor. *The electrophysiological efect of cannabidiol on hERG current and in guinea-pig and rabbit cardiac preparations.* SCIENTIFIC REPORTS 10: 1 Paper: 16079, 9 p. (2020), doi: https://doi.org/10.1038/s41598-020-73165-2. (**IF:** 4.379, **D1**)

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X.) A. Regev, H. Takacs, A.S. Farkas, F. Rarosi, **A. Polyak,** H. Papp, E. Ivany, J.GY. Papp, A. Varro, A. Farkas. *Application of ventricular tachyarrhythmia definitions of the updated lambeth conventions provides incompatibility with earlier results, masks antifibrillatory activity and reduces inter-observer agreement.* JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 70: 1 pp. 37-49., 13 p. (2019), doi: 10.26402/jpp.2019.1.03. (**IF:** 3.011, **Q2**)

XI.) Papp Henriett, Sarusi Annamária, Farkas Attila, **Polyák Alexandra**, Papp Gyula, Varró András, Farkas András. *Repolarizációs tartalékszűkítésen alapuló új proaritmia-modell izolált tengerimalac-szívben.* CARDIOLOGIA HUNGARICA 47: Suppl. G pp. G15-G21. 7 p. (2017), doi: http://doi.org/10.26430/CHUNGARICA.2017.47.suG.15

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1. INTRODUCTION

Physical activity has substantial cardiovascular benefits, improving overall health and reducing the risk of chronic diseases, while excessive exercise may have adverse effects. The U-shaped model illustrates that while moderate exercise has many benefits, engaging in extreme intensity activities may result in detrimental effects on cardiovascular health. This is particularly relevant for those competitive athletes who may have underlying cardiovascular conditions, highlighting the importance of a nuanced approach to balance the advantages of exercise with potential risks.

Elite athletes experience unique heart adaptations, including increased left ventricular size, wall thickness, cardiac mass, and stroke volume, to enhance cardiac performance during demanding activities. These adaptations vary depending on the type and frequency of athletic activities, categorized as "isometric" and "isotonic." However, recent research has uncovered more complex geometric patterns in the athlete's heart, challenging previous assumptions and warranting further investigations. Elite athletes also display distinct cardiac electrical adaptations, with bradycardia and increased heart rate variability, indicative of enhanced autonomic control. However, recent studies challenge the understanding of bradycardia in elite athletes, suggesting it may be related to specific factors, specifically the downregulation of channels conducting the cardiac pacemaker current, rather than changes in cardiac autonomic regulation. However, more research is needed to confirm these findings in humans.

Sudden cardiac death (SCD) in athletes, although rare, occurs more frequently than in non-athletes. The likelihood of SCD is influenced by specific risk factors, such as gender, race, and the type of sport, and is often associated with structural and electrical abnormalities. The "arrhythmic triangle" concept suggests a complex interplay of substrates, triggers, and arrhythmia-promoting modulators making athletes susceptible to life-threatening arrhythmias. Furthermore, various mechanisms, including inherited arrhythmia disorders, electrolyte imbalances, autonomic nervous system dysregulation, certain drugs, and performance-enhancing substances, may collectively contribute to arrhythmias in athletes. This multifactorial perspective challenges the notion that top athletic hearts are always models of health and emphasizes the need for ongoing research at both *in vivo* and cellular levels.

Studying sports-related SCD in humans is challenging, emphasizing the need for fundamental research into athletes' cardiovascular adaptations. Recent animal studies have revealed electrophysiological changes due to high-intensity exercise, including atrial fibrillation, bradycardia, and atrioventricular node dysfunction. Additionally, fibrotic changes

in the hearts of rodents following intense exercise have been associated with an increased risk of arrhythmias. However, it is crucial to note that rodent models have limitations in mirroring human cardiac function. To address this, non-rodent animal models, such as rabbits and dogs, have been selected to better mimic human cardiac properties.

2. AIMS OF THE STUDY

The present study was designed to develop and characterize animal models that closely mimic human cardiac physiology. Its primary aim is to enhance our understanding of the physiological effects of chronic vigorous exercise on cardiac structure and function within the athlete's heart. Furthermore, special attention was given to identifying potential adverse changes in myocardial structure and function and increased arrhythmia sensitivity in both *in vivo* and *ex vivo* settings.

The primary aims of this study are as follows:

I. To gain a comprehensive understanding of the complex electrophysiological changes resulting from prolonged intense exercise, particularly in non-rodent species that closely resemble the human heart in terms of electrophysiological properties.
II. To investigate potential adverse myocardial morphological and functional alterations induced by sustained intensive exercise training.
III. To assess whether prolonged intense exercise leads to increased susceptibility to arrhythmias in both *in vivo* and *ex vivo* settings.
IV. To evaluate the influence of chronic anabolic steroid treatment on cardiac structure and function in animals subjected to long-term exercise regimens.

3. MATERIALS AND METHODS

3.1 General methods, experimental set-up, and training protocols

Animal maintenance and research were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All procedures involving animals were approved by the Ethical Committee for the Protection of Animals in Research of the University of Szeged, Szeged, Hungary (approval numbers: I-74-15-2017 and I-74-24-2017) and by the Department of Animal Health and Food Control of the Ministry of Agriculture and Rural Development (authority approval numbers: XIII/3330/2017 and XIII/3331/2017) and conformed to the rules and principles of the 2010/63/EU Directive.

New Zealand white rabbits (aged 11 months, 3.5-4.0 kg) and mongrel dogs (aged 12-18 months, 7.0-7.5 kg) of both sexes were randomly assigned to sedentary (SED) and exercised

(EX) groups. For mongrel dogs, a doping (DOP) group received testosterone undecanoate treatment. For beagle dogs, a more intense treadmill exercise program was implemented. Beagle dogs (both sexes, 9-15 kg) were divided into sedentary (SED) and trained groups (TRN), with the latter undergoing a 16-week training program. Training included various running sessions and controlled intensity levels. Running sessions were conducted using custom treadmill systems for both species, with training sessions lasting 16 weeks.

3.2 Echocardiography measurements

Two-dimensional M-mode and Doppler echocardiography was performed to evaluate various cardiac parameters in both rabbits and dogs. Left atrial volume (LAV), left atrial volume index (LAV_i), left ventricular end-systolic and diastolic diameters (LVESD and LVEDD), wall thickness parameters (left ventricular posterior wall, LVPW and interventricular septum, IVS), left ventricular mass (LVM), and left ventricular mass index (LVM_i) were assessed. Ejection fraction (EF) was calculated using the Teicholz formula.

3.3 Electrocardiography measurements

ECG measurements were conducted using various methods for rabbits and dogs. ECG intervals, such as RR, PQ, QRS, QT, and T_{peak}-T_{end} (T_pT_e), were measured from recorded sinus beats. Heart rate was derived from the RR interval. The heart rate-corrected QT interval (QT_c) was calculated to eliminate the influence of heart rate. Beat-to-beat variability and instability of RR and QT intervals were also assessed. Arrhythmia incidence was evaluated using established definitions for various arrhythmias. In conscious dogs, the heart rate response to parasympatholytic agent atropine was also evaluated. Additionally, in trained conscious dogs, dofetilide, a class III antiarrhythmic agent, was administered to assess its potential to prolong the QT interval and test proarrhythmic sensitivity.

3.4 Open-chest arrhythmia provocation in anaesthetised canines

Under pentobarbital anaesthesia, ventricular arrhythmia susceptibility was assessed through consecutive ventricular burst pacing with varying pacing durations, delivered epicardially into the left ventricle at a three-fold threshold level. The incidence of induced arrhythmias was compared between the control and trained groups, allowing for an evaluation of arrhythmia susceptibility in response to the training regimen.

3.5 Conventional microelectrode techniques in canine hearts

In heavily trained canine hearts, action potentials were recorded in left ventricular papillary muscle preparations obtained from the hearts of the trained and sedentary dogs using the conventional microelectrode techniques.

3.6 Patch-clamp measurements in canine hearts

Ventricular myocytes were enzymatically dissociated, and the following ionic currents were recorded: L-type calcium current (I_{CaL}), the inward rectifier (I_{K1}), the transient outward (I_{to}), the rapid (I_{Kr}) and the slow (I_{Ks}) delayed rectifier potassium currents, the late sodium current (I_{NaL}), and the Na^+/Ca^{2+} exchanger current (I_{NCX}). Single cell action potentials were measured using the perforated patch-clamp technique on isolated left ventricular myocytes from both trained and sedentary canines. Action potential duration was measured at 90 % repolarization (APD_{90}) and short-term APD variability (STV-APD) was calculated based on 30 consecutive action potentials.

3.7 Western blot analysis of KChIP2 and Kv4.3 proteins in canine hearts

Membrane fractions from canine left ventricular myocardial samples were analysed for KChIP2 and Kv4.3 proteins. Band densities were measured after immunolabelling. Equal loading was confirmed by GAPDH labelling.

3.8 Immunocytochemistry of KChIP2, Kv4.3, and HCN1, HCN2, and HCN4 proteins in canine hearts

Cardiomyocytes from both trained (TRN) and sedentary (SED) dog left ventricular tissue were isolated and immunolabelled for KChIP2, Kv4.3, HCN1, HCN2, and HCN4 proteins. Images were captured using a laser scanning confocal microscope, and quantitative analysis was performed.

3.9 Gene expression of fibrosis markers in rabbit hearts using real-time qRT-PCR

RNA was extracted from left ventricular free wall samples of exercised rabbits and sedentary controls. Real-time PCR was performed, and the expression levels of genes encoding fibrotic biomarkers, including TGF-β, FN-1, COL1A1, COL3A1, MMP-2, and TIMP-1, were analysed.

4. RESULTS

4.1 Sustained exercise-induced cardiac hypertrophy and fibrosis

The 16-week training program resulted in significant cardiac adaptations, with more pronounced effects observed in vigorously trained dogs. Endurance exercise training led to left ventricular dilation and enlargement without a concurrent increase in ventricular wall thickness in both rabbits and dogs. Ejection fraction and fractional shortening did not differ significantly among the groups, and the influence of steroids on cardiac morphology was not prominent. After 16 weeks of more vigorous endurance training in the TRN dog group, they exhibited left atrial enlargement and left ventricular hypertrophy. Trained dogs also had a greater end-diastolic left ventricular volume, indicating the left ventricle's ability to hold more blood during the filling phase. Autopsy results in trained canine hearts confirmed cardiac hypertrophy, along with increased fibrosis levels in the left ventricles compared to sedentary hearts, consistent with relative gene expression results in exercised rabbit hearts.

4.2 The effect of training on heart rate and its variability in conscious canines and rabbits, and on spontaneous beating rate in isolated canine right atrial tissue preparations

In our initial experiments, both trained dogs and rabbits showed prolonged mean RR intervals, indicating training-induced bradycardia. Additionally, beat-to-beat variability parameters suggested vagal enhancement in both trained models. All findings were more pronounced in heavily trained canines. Examining intrinsic beating rates in isolated atrial tissue further supported the presence of bradycardia in trained dogs, independent of vagal tone enhancement. The moderate increase in heart rate observed in the trained animals was consistent with the findings of spontaneous heart rate measurements.

4.3 ECG changes and increased proarrhythmic response following long-term sustained training

In our initial experiments, the training protocol did not result in significant changes in depolarizing PQ and QRS intervals or heart rate-corrected QT intervals (QT_c) in rabbits and dogs. Minor increases in beat-to-beat QT variability values were observed in exercised rabbits, but not in dogs. However, heavily trained dogs subjected to a 16-week training regimen displayed substantial prolongation of RR, PQ, QT, QTc, and T_pT_e intervals, along with widening of the QRS complex. This prolonged QT interval was associated with increased QT interval variability (STV-QT), indicating greater repolarization dispersion compared to

sedentary controls. Moreover, heavily trained dogs exhibited a higher incidence of ventricular beats, including ventricular escape beats and premature beats, during ECG recordings at rest. During electrical burst stimulation in open-chest, anesthetised dogs, ventricular fibrillation was induced more frequently in trained dogs compared to sedentary controls.

4.4 Repolarization sensitivity to the proarrhythmic agent dofetilide in conscious canines

To assess the repolarization sensitivity of athletes' hearts, dofetilide, an I_{Kr} inhibitor, was administered to dogs from both the exercise training (EX, DOP, and SED groups) and heavily intense training (TRN and SED groups) protocols following the 16-week training program. Dofetilide significantly increased the heart rate-corrected QT interval (QT_c) in all groups, with a more pronounced effect in trained animals. There were no meaningful differences in QTc intervals between the exercised (EX) and doping (DOP) groups. The percentage of QT_c prolongation calculated from baseline and post-dofetilide values did not differ significantly among the groups, indicating an additive effect of training and dofetilide on QT_c lengthening. Dofetilide treatment increased beat-to-beat variability and instability parameters in all groups, with a slightly more pronounced repolarization inhomogeneity in heavily trained animals compared to controls. While dogs subjected to less intensive training showed a modest occurrence of ventricular premature beats during dofetilide perfusion, no significant intergroup differences were observed. However, heavily trained dogs exhibited an elevated frequency of ventricular beats and more complex arrhythmias, increasing the risk of life-threatening arrhythmia development.

4.5 Influence of prolonged training on cardiac APD and its short-term variability in left ventricular preparations of heavily trained and sedentary canines

Cardiac action potential duration (APD_{90}) was similar in isolated left ventricular papillary muscles (subendocardial origin) between groups. However, in enzymatically isolated left ventricular single myocytes (midmyocardial origin), TRN dogs showed prolonged APD_{90} and increased STV-APD compared to SED animals.

4.6 Effects of chronic sustained training on various transmembrane ionic currents in canine left ventricular myocytes

The magnitude of the I_{to} current was significantly smaller in myocytes obtained from the trained dogs compared to the sedentary animals. However, there were no significant differences in the magnitudes of the I_{NaL}, $I_{NCX,}$ L-type $I_{Ca,}$ I_{K1}, I_{Kr}, and I_{Ks} currents.

4.7 The relative density of transmembrane Kv4.3 and KChiP2 proteins in trained and sedentary canine hearts

The investigation into the molecular basis of the I_{to} current in TRN dog hearts found no significant differences in the expression of Kv4.3 and KChiP2 proteins, compared to SED dog hearts. This suggests that the decrease in the I_{to} current magnitude may be related to other accessory proteins or post-translational modifications in ion channel proteins.

4.8 HCN4 channel upregulation in left ventricles after sustained training

Immunocytochemistry of left ventricular myocytes in TRN dog hearts showed significantly increased HCN4 protein expression compared to SED dogs, while no differences were observed in the expression of HCN1 and HCN2 proteins.

4.9 Gene expression of fibrosis biomarkers in rabbit hearts after 16 weeks of training

After 16 weeks of endurance training in rabbits, the relative gene expression of fibrosis-related markers showed a significant increase in COL3A1, MMP-2, and TIMP-1 in the exercise group compared to the sedentary group. COL1A1 and FN-1 expression had a minor, nonsignificant increase, and TGF-ß expression remained similar in both groups.

4.10 Testosterone levels in canines

DOP dogs had higher serum testosterone levels compared to EX and SED dogs, with no significant differences in other examined laboratory parameters, including electrolytes, renal, and hepatic functions.

5. DISCUSSION

In this study, long-term endurance exercise was conducted on two non-rodent species with cardiac morphological, electrophysiological, and autonomic neural properties similar to humans. These animal models provided important insights into the electrophysiological characteristics of the athlete's heart and its associated elevated risk of ventricular arrhythmias.

The main findings of the study include:

1) Successful development of training protocols that allowed us to replicate the athlete's heart phenotype in two distinct, human-relevant animal models.
2) Sustained endurance training in dogs resulted in athlete's heart characteristics, including left ventricular hypertrophy and increased atrial and ventricular volumes, while less intense training protocols in rabbits and dogs did not affect ventricular wall thickness, emphasizing the impact of training intensity on cardiac remodeling.

3) Sustained endurance training increased heart rate variability, indicating an increased parasympathetic tone, and decreased resting heart rate in both whole animal and *in vitro* experiments. This suggests that factors beyond vagal tone contribute to training-induced bradycardia. A hypothesis was further validated by experiments with parasympatholytic agents in dogs.
4) Sustained endurance training consistently prolonged cardiac repolarization, resulting in *in vivo* ECG QT_c lengthening and APD prolongation along with reduced I_{to} current density in cellular measurements. These findings were associated with increased variability of cardiac repolarization, indicating impaired repolarization following intensive training.
5) No significant differences were observed in the expression of Kv4.3 alpha or KChIP2 beta accessory channel proteins between SED and TRN dog hearts, implying that reduced I_{to} current density may be influenced by other factors.
6) Trained dogs from both experiments exhibited higher sensitivity to the QT_c prolongation induced by the class III antiarrhythmic agent dofetilide, along with an increase in the number and complexity of arrhythmias. However, the combined effect of training and dofetilide on QT_c lengthening was additive rather than superadditive.
7) Sustained endurance training increased the risk of ventricular fibrillation during electrical stimulation, indicating higher susceptibility to arrhythmias in trained dog hearts.
8) Sustained endurance training upregulated HCN4 channels in dog ventricular myocardium, potentially promoting arrhythmogenesis by enhancing ectopic electrical activity.
9) Sustained endurance training increased fibrosis in dog ventricular myocardium and elevated the expression of fibrotic genes in rabbit hearts, potentially contributing to arrhythmia sensitivity by creating arrhythmogenic substrates.
10) Administration of testosterone at a specific dose, along with a standardized endurance exercise program, did not induce an increase in cardiac muscle mass and had no significant impact on other structural and *in vivo* repolarization parameters in dogs.

5.1 Animal models of the athlete's heart: Insights from rabbits and canines

This study aimed to investigate the potential cardiac effects of high-intensity exercise in elite athletes. Due to limitations in studying these effects in humans, the research used animal models. It compared two animal models, rabbits and canines, to better understand the cardiac adaptations to endurance training. Both rabbit and canine models provided essential cardiac electrophysiological information on the athlete's heart. While the rabbit model offers

advantages in terms of cost, time, and resource-effectiveness compared to larger animal models, the canine model represents the most human-relevant data.

5.2 Cardiac morphological remodelling in response to chronic exercise

The study aimed to investigate cardiac morphological changes resulting from different exercise modalities in animal models. The response to exercise can vary depending on the specific nature and frequency of the physical activity. Therefore, it was essential to assess how the applied exercise affected cardiac morphology in the experimental models. In endurance-trained rabbits and in exercised dogs, we found increased left ventricular end-diastolic and aortic root diameters, indicating cardiac volume overload. This is consistent with observations in competitive male endurance athletes who also showed greater aortic and left ventricular dimensions. However, there were no significant changes in interventricular septum or posterior wall thickness, and left ventricular contractile function remained normal. In the heavily trained canine model, structural changes included left ventricular chamber enlargement and increased wall thickness in the trained group. However, similar to human data, the ejection fraction remained unchanged. These findings are in line with exercise-induced cardiac remodelling seen in elite endurance athletes. Notably, the study also found that the left atrial volume and left atrial volume index increased in the trained dogs, similar to what is observed in highly trained athletes. These atrial changes could potentially contribute to arrhythmias like atrial fibrillation, which is common in athletes. The observed variations in wall thickness changes may be influenced by the nature and duration of the physical activity they performed.

5.3 The complex mechanism of bradycardia

This study emphasizes the complexity of bradycardia development, involving both increased vagal tone and intrinsic alterations in the sinoatrial node. While the prevailing interpretation attributes bradycardia to increased vagal tone in both athletes and animal exercise studies, recent researches introduce a novel perspective, proposing electrical remodelling of the sinoatrial node (SAN), specifically the reduction in hyperpolarization-activated "funny" current (I_f) density and the remodeling of the underlying HCN4 ionic channel. In the present study, sinus bradycardia was observed in isolated right atrial preparations from trained dog hearts after the termination of autonomic system influence. Additionally, the moderate increase in heart rate after the administration of a parasympatholytic atropine infusion in the exercised dog group suggests that long-term endurance training may lead to intrinsic adaptations in the sinoatrial node in addition to increased vagal tone. Furthermore, the increased beat-to-beat variability of cycle length and first-degree atrioventricular block indicate an important contribution of

enhanced vagal tone. These parameters reflect parasympathetic activity, which is a common feature among athletes with high aerobic resistance. In summary, the study suggests that changes in cardiac autonomic regulation and intrinsic SAN changes are parallel responses to vigorous exercise, even though the precise underlying mechanisms remain unexplored.

5.4 Mechanistic basis of arrhythmias in the athlete's heart model

Exercise-induced bradycardia results in prolonged action potential duration and increased dispersion of cardiac repolarization, posing an arrhythmia risk, especially when combined with hypokalaemia. This phenomenon potentially contributes to an increase in the arrhythmic substrate factor within the classical "arrhythmic triangle" concept, indicating that arrhythmias occur when specific combinations of substrates, triggers, and arrhythmia-promoting modulators are present. Moreover, bradycardia resulting in longer diastolic intervals may enhance the probability of spontaneous diastolic depolarization reaching the firing threshold, potentially acting as an arrhythmia trigger. Vagal activity alterations could also trigger life-threatening arrhythmic episodes. In canine mid-myocardial myocytes a reduction in the I_{to} current was observed, potentially contributing to repolarization impairment and arrhythmogenic substrates. The incidence of induced ventricular fibrillation also increased in trained animals, possibly linked to the observed HCN4 protein overexpression in the ventricle, facilitating enhanced ectopic electrical activity and promoting arrhythmogenesis. Trained rabbits and dogs exhibited increased fibrous tissue formation in the left ventricle, which could disrupt normal cardiac function and increase the risk of arrhythmias.

5.5 Impacts of exercise on ventricular repolarization in the canine and the rabbit athlete’s heart models

Both animal models, with the most prominent alterations seen in heavily trained dogs, exhibited impaired cardiac repolarization following chronic exercise, characterized by longer QT_c intervals, prolonged APD in canine myocytes, and increased repolarization variability parameters *in vivo* and *in vitro*. These changes suggest increased spatial and temporal dispersion of repolarization, contributing to arrhythmia development. The use of dofetilide, an I_{Kr} channel blocker, further demonstrated repolarization impairment following intense training. In the canine model, a reduction in the I_{to} current in midmyocardial myocytes may be responsible for this impairment, consistent with prior research correlating reduced I_{to} current with APD prolongation in failing hearts. Other key ionic currents involved in repolarization did not significantly differ between groups in the canine model. The lower I_{to} current density in trained dogs did not appear to result from reduced expression of Kv4.3 or KChIP2 proteins, suggesting

that other accessory proteins and intracellular signalling pathways may influence I_{to} function. It is important to note that repolarization changes induced by training, though mild, may accumulate with other factors, potentially providing a substrate for arrhythmias.

5.6 Steroid-induced cardiovascular risk among endurance athletes

Anabolic-androgenic steroid (AAS) misuse poses significant cardiovascular risks, including elevated blood pressure, adverse lipid profiles, thrombotic events, cardiac hypertrophy, and impaired cardiac repolarization. These factors can collectively contribute to arrhythmias, an elevated risk of heart failure, and even sudden cardiac death. We revealed relatively mild changes in mood, libido, and body muscle mass, with no significant changes in cardiac parameters in animals that received testosterone treatment alongside an endurance exercise program. This might be due to the relatively low dosage used in the research, as real-life athletes might use higher doses of AAS. Combining AAS with other drugs and variations across different sports disciplines can also impact the results.

7. CONCLUSION

Vigorous endurance training specific to running was associated with prolonged cardiac repolarization and increased susceptibility to arrhythmias in animal models mimicking the human athlete's heart. While exercise is generally beneficial, such training may pose additional risks, particularly for individuals with underlying health conditions. This research underscores the complexity of arrhythmia development in athletes, providing valuable insights for tailoring exercise regimens and medication choices in this population. Further research is necessary to translate these findings from animals to human athletes accurately.

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