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ORIGINAL RESEARCH

Long-term endurance training-induced cardiac adaptation in new rabbit and dog animal models of the human athlete's heart

Alexandra Polyák[1,2], Péter Kui[3], Nikolett Morvay[3], István Leprán[3], Gergely Ágoston[4], Albert Varga[4], Norbert Nagy[2,3], István Baczkó[3], András Farkas[1], Julius Gy. Papp[2,3], András Varró[2,3,*] and Attila S. Farkas[1]

[1] Second Department of Medicine and Cardiology Centre, University of Szeged, Szeged H-6725, Hungary

[2] MTA-SZTE Research Group for Cardiovascular Pharmacology, Hungarian Academy of Sciences, Szeged H-6720, Hungary

[3] Department of Pharmacology and Pharmacotherapy, University of Szeged, Szeged H-6720, Hungary

[4] Institute of Family Medicine, University of Szeged H-6720, Hungary

*Correspondence to András Varró: varro.andras@med.u-szeged.hu

Sudden cardiac death in athletes is rare and most often unexpectable. For a better understanding of cardiac remodeling, this study presents the effects of chronic vigorous exercise on cardiac structure and electrophysiology in new rabbit and dog athlete's heart models. Rabbits and dogs were randomized into sedentary ('Sed'), exercised (subjected to 16 weeks chronic treadmill exercise ('Ex') groups, and a testosterone-treated ('Dop') group in dogs. Echocardiography and electrocardiogram were performed. Proarrhythmic sensitivity and autonomic responses were tested in conscious dogs. 'Ex' animals exhibited left ventricular enlargement with bradycardia (mean RR in 'Ex' vs. 'Sed' rabbits: 335 ± 15 vs. 288 ± 19 ms, $p \le 0.05$, and in 'Dop' vs. 'Ex' vs. 'Sed' dogs: 718 ± 6 vs. 638 ± 38 vs. 599 ± 49 ms) accompanied by an increase of heart rate variability in both species (e.g. SD RR in 'Ex' vs. 'Sed' rabbits: 3.4 ± 0.9 vs. 1.4 ± 0.1 ms, $p \le 0.05$, and in 'Dop' vs. 'Ex' vs. 'Sed' dogs: 156 ± 59 vs. 163 ± 44 vs. 111 ± 49 ms) indicating an increased vagal tone. A lower response to parasympatholytic agent atropine and more pronounced QT_c interval lengthening after dofetilide challenge were found in 'Ex' and 'Dop' dogs compared to the 'Sed' group. No morphological and functional changes were found after chronic steroid treatment in dogs. The structural-functional findings share more similarities with human athlete's heart. Slight repolarization sensitivity in the exercised dogs may indicate an increased risk of arrhythmias in athletes under different circumstances. These animal models might be useful for the further investigations of the cardiovascular effects of competitive training.

Keywords

Endurance exercise; cardiac structure; cardiac function; remodeling; echocardiography; electrophysiology

1. Introduction

Athletes are perceived as the healthiest segment of society, however, tragic sudden cardiac deaths (SCD) involving young, seemingly healthy competitive athletes were reported several times in the recent years. While SCD among athletes is rare (approximately 1:50.000-1:100.000), its incidence is still two to four times more frequent in athletes compared to their nonathletic counterparts (Marijon et al., 2011). Numerous structural, electrical, and acquired cardiovascular abnormalities, e.g. hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy and atherosclerotic coronary artery disease in athletes older than 35 years of age (Corrado et al., 2003; Maron et al., 2009), are considered to lead SCD under different circumstances. However, in the 3-6% of cases the real cause of SCD remains unclear (Maron et al., 2009).

According to Maron et al, SCD occurs more frequently in elite football and basketball players (Maron et al., 1996) suggesting that individuals participating in sports of high dynamic and low isometric intensity are at higher risk of death. It is hypothesized that ventricular arrhythmias, e.g. Torsades de Pointes (TdP) acting on an arrhythmogenic substrate, might have an important role in SCD cases (Varro and Baczko, 2010). Postulated contributory mechanisms include genetic defects, electrolyte alterations, autonomous nervous system imbalances and performance-enhancing drugs associated with exercise. These factors together can increase the cardiac repolarization inhomogeneity leading to life-threatening arrhythmias (Farkas and Nattel, 2010; Varro and Baczko, 2010).

Evaluation of athletes poses diagnostic difficulties, particularly differentiating physiological adaptation with associated electrocardiographic and echocardiographic changes attributed to 'athlete's heart', from cardiac pathology that may result in SCD. Thus, there is a strong need for more basic research to assess the physiological adaptation of cardiovascular system and the regulating mechanism exerted by the autonomic nervous system on cardiovascular functions in athletes. Since, the examination of electro-

physiological features of athlete's heart in young athletes are understandably limited, the purpose of our study was to determine the effect of chronic high dynamic exercise-induced cardiac adaptation in those non-rodent species that are electrophysiologically relevant to the human heart. An attempt was made to estimate the impact of the chronic anabolic steroid treatment on cardiac structure and function in long-term exercised animals. This study also compares the cardiovascular effects of exhaustive endurance training in experimental athlete's heart models.

2. Materials and methods

2.1. Animals

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 using animals were approved by the local ethics committee (including the Ethical Committee for the Protection of Animals in Research at University of Szeged, Hungary) and conformed to the rules and principles of the 2010/63/EU Directive.

2.2. Experimental protocol

New Zealand white rabbits, weighing 3.5-4.0 kg (1st set of experiment) and mongrel dogs, weighing 7.0-7.5 kg (2nd set of experiment) from either sex were randomized into sedentary ('Sed', rabbit n = 7; dog n = 2) and exercised ('Ex', rabbit n = 7; dog n = 2) groups. In the 2nd set of experiment a doping ('Dop', dog n = 2) group was also applied: long-acting anabolic androgen steroid (AAS) medication, testosterone undecanoate (Nebido, Bayer AG, Germany) was administered by intramuscular injection at a dosage of 14.3 mg per kg of body weight in intervals of four weeks. Rabbits and dogs from the exercised and control groups were chosen from the same age. New Zealand White rabbits were 11 months old and mongrel dogs were 12 months old at the beginning of the long-term endurance training protocol. The training sessions of 'Dop' group were identical to 'Ex' group, and therefore the effects of testosterone could not be attributed to diverse training conditions. During the 2nd set of experiment blood collection was performed at every fourth week from the cephalic or saphenous vein and the levels of serum testosterone, electrolytes, blood chemistry and quantitative blood count parameters were measured. The complete experimental protocol is shown in Fig. 1.

Running sessions were performed on a self-developed treadmill system, with two separated corridors for the animals and a control panel to modulate speed intensity. 'Ex' and 'Dop' animals underwent a 16-week-long training session, while 'Sed' group did not participate in the training. The protocol started with a 2-week-long warm-up period, thereafter animals were trained for 5 days a week with 20 minutes daily running sessions at speed 2.5-3 km·h^{-1} for 16 weeks (1st set of experiment, rabbits) and with 2×90 minutes at speed 6-10 km·h^{-1} for 16 weeks (2nd set of experiment, dogs). The training intensity was maintained with the use of 5% to 12% inclination. The training protocol was tested in preliminary experiments and set to the maximum level which could be performed without distress yet.

2.3. Echocardiography

Echocardiography was performed at 0 and 16 weeks of the training protocol. M-mode parasternal long axis view was applied using 11.5 MHz transducer (GE 10S-RS, GE Healthcare, Chicago, IL, USA), connected to an echocardiographic imaging unit (Vivid S5, GE Healthcare, Chicago, IL, USA). All parameters were analysed by an investigator in a randomised and blinded manner. Left ventricular internal diameter during systole (LVIDs) and diastole (LVIDd), thickness of the left ventricular posterior wall (LVPW) and interventricular septum (IVS) were measured in M-mode images. Fractional shortening was calculated as [(LVIDd-LVIDs)/LVIDd] × 100.

2.4. Electrocardiography

At 0 and at 16 weeks ECGs were recorded simultaneously with National Instruments data acquisition hardware (PC card, National Instruments, Austin, TX., U.S.A.) and SPEL Advanced Haemosys software (version 3.26, Experimetria Ltd. and Logirex Software Laboratory, Budapest, Hungary).

In anaesthetised rabbits the ECG recording was made with needle electrodes that placed subcutaneously in all four limbs according to Farkas et al. (Farkas et al, 2004). In conscious dogs the ECG was measured using precordial leads. The ECG was digitized and stored on a computer for later analysis. RR, PQ, QRS, QT and T$_{peak}$-T$_{end}$ intervals were measured by manual positioning on screen markers of 40 consecutive sinus beats at the 10th minute after initiation of the recording, then mean values were calculated. Heart rate was calculated from the RR interval. As QT interval is influenced by the heart rate, baseline data for ventricular heart rates and QT intervals were used to determine the relationship between the RR interval and the QT interval in sinus rhythm according to (Farkas et al., 2003; Kui et al., 2016). These data were obtained from 14 *in vivo* rabbits and from 6 dogs. Forty consecutive QT intervals were measured together with the corresponding RR intervals. Simple linear regression revealed a positive correlation between QT and RR intervals in rabbits (QT$_{rabbit}$ = 0.354RR + 51.7) and dogs (QT$_{dog}$ = 0.04RR + 188.5). The equations were rearranged to allow the calculation of the rate-corrected QT interval in rabbits at an RR interval of 295 ms (i.e. a ventricular rate of 203 beats·min^{-1}) using the formula QT$_{cx}$ = QT$_x$-0.354 (RR$_{x-1}$-295) and in dogs and at an RR interval 613 ms (i.e. a ventricular rate of 98 beats·min^{-1}) using the formula QT$_{cx}$ = QT$_x$-0.04 (RR$_{x-1}$-613) (Fig. 2A and Fig. 2B). With these equations, plotting QT$_{c-rabbit}$ and QT$_{c-dog}$ against the corresponding RR interval produces a regression line with a slope of zero (Fig. 2C and Fig. 2D), indicating that these corrections remove the influence of heart rate.

2.4.1. Measurement of the beat-to-beat variability and instability of the ECG intervals

Beat-to-beat variability and instability (BVI) parameters of the RR, PQ, QRS, QT and T$_{peak}$-T$_{end}$ intervals (e.g. the 'root mean square of the successive differences', the 'standard deviation of the successive differences', the 'short-term variability', 'long-term variability', 'short-term instability', 'long-term instability', 'total instability' and 'instability' of the ECG intervals) were derived from 40 consecutive sinus beats as described previously (Farkas et al., 2009; Sarusi et al., 2014; Vincze et al., 2008).

2.5. Dofetilide and atropine challenges in vivo in dogs

In conscious dogs, class III antiarrhythmic agent dofetilide perfusion was performed at 0 and 16 weeks for assessing the QT in-
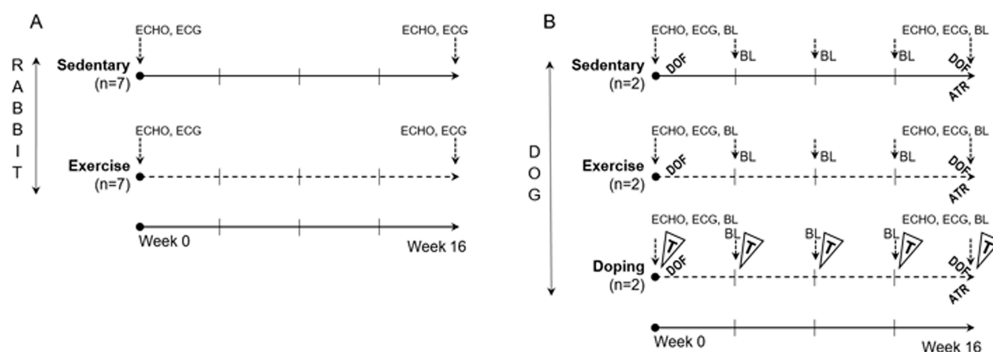
Figure 1. Experimental protocol in rabbits (A) and dogs (B). Continuous line, Do not participate in training sessions ('Sed' groups); Dotted line, Participate in training sessions ('Ex' and 'Dop' groups); ECHO, Echocardiography; ECG, Electrocardiography; BL, Blood collection; DOF, Dofetilide challenge, (0.035 mg/kg iv.); ATR, Atropine sulfate challenge, (0.04 mg/kg iv.); T, Testosterone-undecanoate treatment, (14.3 mg/kg im.).
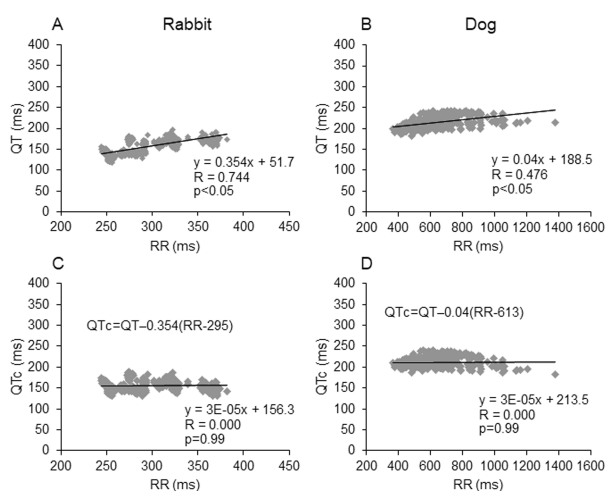

Figure 2. Correlation between individual values of the QT and RR intervals in rabbit (A) and in dog (B) hearts *in vivo*. Correlation between individual values of the 'rate-corrected' QT intervals and the RR intervals in rabbit (C) and in dog (D) hearts *in vivo*. Rabbit panels contain 560 baseline data points obtained from 14 rabbits *in vivo*. Dog panels contain 480 baseline data points obtained from 6 dogs *in vivo*. QT_c, heart rate-corrected QT interval.

terval prolonging potential and testing the proarrhythmic sensitivity before and after the long-term training protocol. Drug-free resting ECG was recorded for 20 minutes. Resting ECG values were determined at the 19th minute after initiation of the recording ('Baseline'; 1 minute before dofetilide treatment). It was followed by a bolus administration of intravenous dofetilide (a selective inhibitor of the rapidly activating inward rectifying component of net delayed rectifier K$^+$ current, 'I_{Kr}') at a dosage of 0.035 mg per kg. ECG intervals were measured 15 minutes after dofetilide administration, in the 40th experimental minute ('Dof' period). Dofetilide was dissolved in dimethyl sulphoxide (DMSO). Dofetilide and DMSO were obtained from Sigma-Aldrich, Inc. (Vienna, Austria).

In conscious dogs at 16th week heart rate response was tested by intravenous administration of the parasympatholythic agent atropine sulfate (Egis Pharmaceuticals PLC, Budapest, Hungary). After recording resting ECGs for 20 minutes ('Baseline' period), 0.04 mg per kg atropine, dissolved in saline, was administered. The mean values of 40 consecutive RR intervals were measured at drug-free control period (T1) and 2, 5, 10, 20 minutes after atropine treatment (T2-T5).

3. Statistics

IBM SPSS Statistics V25 software package was used for statistical analysis. Continuous data were expressed as mean ± standard error of the mean (S.E.M.). Student's t-test was applied to estimate whether there is a statistically significant difference between the means in independent groups. Data were considered statistically significant when $p < 0.05$.

4. Results

4.1. Structural and functional echocardiographic parameters

The 16-week endurance-training program resulted in significantly greater internal end-diastolic diameter of the left ventricle (LVIDd) in the 'Ex' rabbit group, and in an increasing tendency in LVIDd in 'Ex' dogs. A moderate internal end-systolic diameter of the left ventricle (LVIDs) increment was also seen in 'Ex' rabbits and 'Ex' dogs (Table. 1). Interestingly, the thickness of the left ventricular posterior wall (LVPW) and the interventricular septum (IVS) did not differ significantly between the groups neither in rabbit nor in dog models (Table. 1). The left ventricular muscle began to dilate and enlarge as a result of endurance exercise training, without an increase in ventricular wall thickness. The ejection fraction (EF) and the fractional shortening (FS) did not indicate any difference between the groups. The complete list of measured echocardiographic parameters is shown in Table. 1.

4.2. Mean ECG depolarization parameters and RR beat-to-beat variability parameters

The ECG showed lengthened mean RR intervals in all exercised groups of both species, which corresponded to training

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Table 1. Echocardiographic cardiac dimensions and performance in rabbits (above) and dogs (below) after 16 weeks endurance exercise. LVIDd and LVIDs, Left ventricular internal diameter in diastole and systole; IVS, Interventricular septum, LVPW, Left ventricular posterior wall; EF, Ejection fraction; FS, Fractional shortening. All values are means ± SEM. * $p < 0.05$ vs. 'Sedentary'.

	Examined Groups	LVIDd (mm)	LVIDs (mm)	IVS (mm)	LVPW (mm)	EF(%)	FS(%)
RABBIT (N=7)	Sedentary	13.6 ± 0.7	9.2 ± 0.7	3.3 ± 0.2	3.2 ± 0.2	64.7 ± 4.1	33.0 ± 3.1
	Exercise	17.5 ± 0.6*	10.6 ± 0.6	3.2 ± 0.2	2.7 ± 0.1	70.7 ± 2.8	37.7 ± 2.4
DOG (N=2)	Sedentary	24.0 ± 3.0	12.5 ± 0.5	7.4 ± 0.3	7.0 ± 0.1	80.4 ± 3.7	47.0 ± 4.2
	Exercise	27.2 ± 0.2	14.9 ± 0.1	7.3 ± 0.6	6.5 ± 0.5	78.8 ± 0.5	45.6 ± 0.5
	Doping	22.5 ± 1.5	13.5 ± 0.5	6.6 ± 0.3	6.5 ± 0.4	72.4 ± 4.5	39.8 ± 4.0

bradycardia, indicating increased vagal tone (Fig. 3A and Fig. 3B). There was no difference in depolarizing PQ and QRS intervals after the completion of the training protocol (Rabbit mean-PQ 'Ex' vs. 'Sed': 74.0 ± 6.5 vs. 67.9 ± 2.0 ms; Dog mean-PQ 'Ex' vs. 'Dop' vs. 'Sed': 90.8 ± 8.0 vs. 95.2 ± 3.2 vs. 91.2 ± 3.5 ms; Rabbit mean-QRS 'Ex' vs. 'Sed': 49.8 ± 4.2 vs. 56.5 ± 5.2 ms; Dog mean-QRS 'Ex' vs. 'Dop' vs. 'Sed': 60.3 ± 3.6 vs. 56.5 ± 0.8 vs. 50.8 ± 9.3 ms).

After 16 weeks the beat-to-beat root mean square (RMS), standard deviation (SD) and total instability (TI) of the 'Ex' RR intervals significantly increased compared to 'Sed' in rabbits (Fig. 4A-C). Similar heart rate variability increasing tendency was found in 'Ex' and 'Dop' dogs (Fig. 4D-F), although significant difference was not found.

4.2.1. Atropine challenge in dogs

Repeated measurements on resting RR intervals during drug-free period (as a first stage before atropine challenge) confirmed the training-induced bradycardia in both 'Ex' and 'Dop' dogs (Fig. 3C). However, continuous atropine infusion could not retract the lengthened the RR values of 'Ex' and 'Dop' groups to the level of RR intervals of 'Sed' group indicating a decreased atropine response after long-term exercise (Fig. 3C).

4.3. Mean and beat-to-beat variability ECG QT parameters of ECG intervals

The mean and the beat-to-beat root mean square (RMS) of the QT intervals were mildly longer at the end of the training protocol in the exercised animals in both sets of experience (rabbit mean-QT 'Ex' vs. 'Sed': 192.6 ± 6.5 vs. 166.7 ± 10.9 ms; RMS-QT: 192.7 ± 6.5 vs. 166.8 ± 10.9 ms; dog mean-QT 'Ex' vs. 'Dop' vs. 'Sed': 219.7 ± 9 vs. 218.2 ± 16.5 vs. 204 ± 2.3 ms; RMS-QT: 219.7 ± 9 vs. 224.8 ± 9.8 vs. 204.0 ± 2.3 ms), although this can be the result of the longer RR intervals due to bradycardia. Accordingly, corrected QT intervals (QT_c) did not differ between the groups after the long-term physical exercise (Fig. 5A and Fig. 5B).

Some beat-to-beat QT variability values were increased in 'Ex' rabbits (e.g. short-term variability (STV): 'Ex' vs. 'Sed': 4.4 ± 0.4 vs. 4.0 ± 0.5 ms; long-term instability (LTI): 5.5 ± 1.1 vs. 4.1 ± 0.4 ms; instability (I): 9.6 ± 1.5 vs. 7.6 ± 0.9 ms), although these parameters did not reach statistical significance. No difference and tendencies were found in QT BVI parameters in dogs (STV: 'Ex' vs. 'Dop' vs. 'Sed': 3.6 ± 0.2 vs. 2.8 ± 0.4 vs. 4.5 ± 0.7 ms; LTI: 4.7 ± 1 vs. 2.6 ± 0.8 vs. 5.3 ± 1.9 ms; I: 7.7 ± 1.5 vs. 5.3 ±
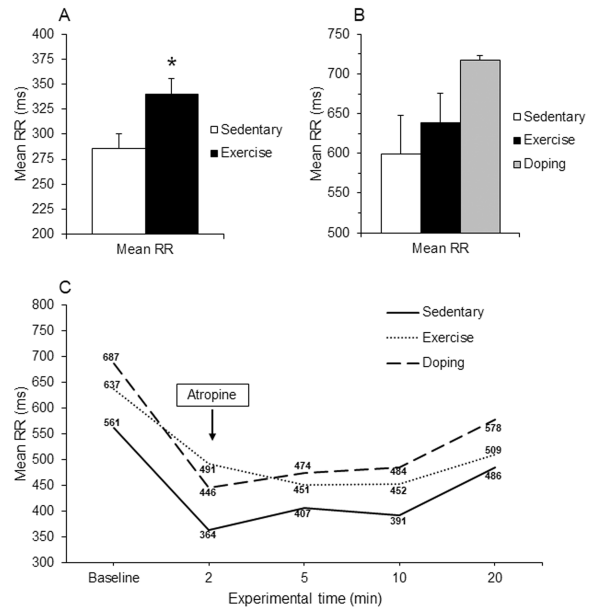

Figure 3. The mean *in vivo* RR intervals in rabbits (A) and dogs (B) and the mean RR intervals in dogs after parasympatholytic atropine challenge (C) at 16th week. All values are means ± SEM. * $p < 0.05$ vs. 'Sedentary'.

2.4 vs. 8.7 ± 3.2 ms).

4.3.1. Repolarization sensitivity to proarrhythmic agent dofetilide in conscious dogs

To examine the repolarization sensitivity of athlete's hearts, I_{Kr} inhibitor was administered to the dogs after the training protocol. As it was expected, dofetilide markedly increased the QT_c interval in each group (Fig. 5C), however, QT_c prolongation was more pronounced in 'Ex' and 'Dop' hearts compared to 'Sed' hearts (Fig. 5C). There was no meaningful difference in QT_c interval between the exercised ('Ex') and doping ('Dop') groups (Fig. 5C).

Despite the marked QT_c interval lengthening effect, no significant difference was found in the beat-to-beat variability and instability parameters of the repolarization after dofetilide treatment (e.g. STV: 'Ex' vs. 'Dop' vs. 'Sed': 4.7 ± 0.6 vs. 4.4 ± 1.0 vs. 4.6 ± 1.0 ms; LTI: 4.8 ± 0.7 vs. 3.5 ± 0.9 vs. 3.6 ± 0.3 ms; I: 9.9 ± 1.9 vs. 6.4 ± 0.7 vs. 7.7 ± 1.3 ms).

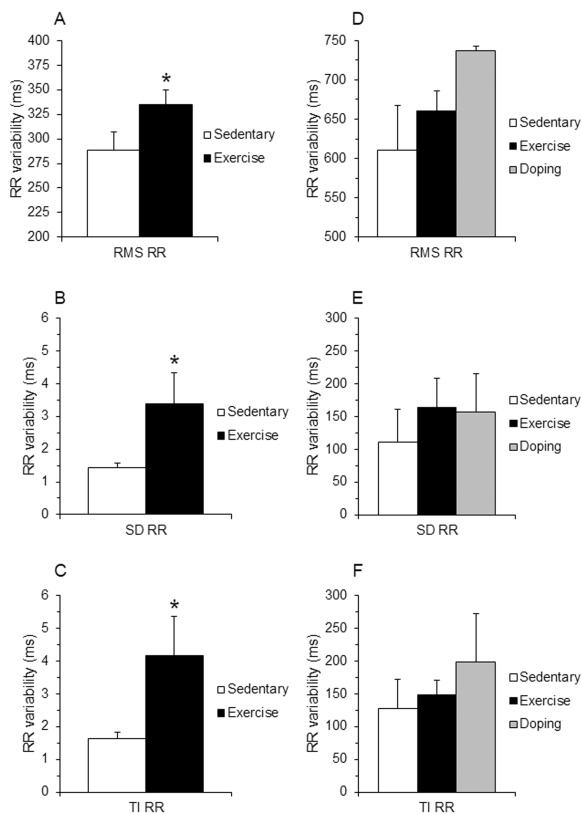During dofetilide perfusion a low number of ventricular pre-

Figure 4. Heart rate variability parameters in rabbits (A-C) and dogs (D-F) at 16th week. Values were derived from 40 consecutive ventricular complexes during sinus rhythm. RMS, root mean square; SD, standard deviation; TI, total instability. All values are means ± SEM. * $p < 0.05$ vs. 'Sedentary'.

Figure 5. The heart rate corrected QT intervals in rabbits (A) and in dogs (B) and the heart rate corrected QT interval changes of dogs after dofetilide challenge ('Dof' period) at 16th week (C). Values were derived from 40 consecutive ventricular complexes during sinus rhythm. QT_c, heart rate-corrected QT interval; Baseline, drug-free values; Dof, values after 15 minutes dofetilide challenge. All values are means ± SEM.

mature beats occurred, but no significant difference was found between the groups. More complex arrhythmias (e.g. Torsades de Pointes) did not develop during dofetilide perfusion in any groups (data not shown).

4.4. Testosterone levels and laboratory parameters in dogs

Testosterone levels were higher in 'Dop' dog blood samples over the 16-week long training period than the testosterone-free dogs ('Dop' vs. 'Ex' vs. 'Sed' in male: 19.4 ± 3.2 vs. 10.2 ± 1.4 vs. 11.2 ± 1.4 nmol/l, $p < 0.05$; in female: 18 ± 4.1 vs. < 0.43 vs. < 0.43 nmol/l, $p < 0.05$).

No significant difference was revealed between the groups in any other laboratory parameters (data not shown). Thus, long-term physical exercise did not influence any assessed internal organ laboratory parameters.

5. Discussion

Long-term endurance exercise was performed on two non-rodent species that might share similar cardiac electrophysiological and autonomic neural properties with humans. Cardiac morphological and functional adaptation signs, including echocardiographic findings of left ventricular enlargement and increased parasympathetic tone leading to decreased resting heart rate and increased heart rate variability were found.
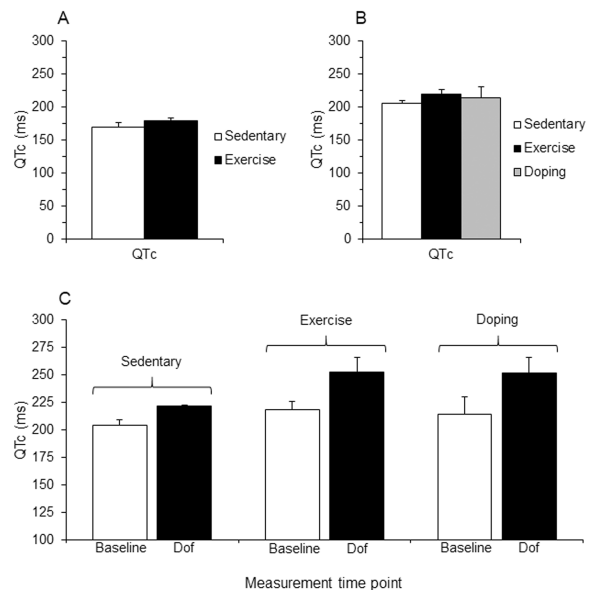
Moderate heart rate sensitivity to the parasympatholytic agent atropine and a tendency of higher sensitivity to the QT_c lengthening dofetilide using a self-calculated QT_c equation was found in dogs during in vivo proarrhythmic challenge. These trends might indicate delayed ventricular repolarization that predisposes to malignant ventricular tachycardia, e.g. Torsades de Pointes.

5.1. Animal models of the human athlete's heart

In animal models a variety of cardiac morphological-functional processes can be studied in vivo and even in vitro at an organ, cellular and molecular level, however the choice of a model needs to be considered carefully, since it vitally affects experimental outcomes.

Small rodents are widely used since they are easier to handle and have shorter gestation period which allow larger sample size (Milani-Nejad and Janssen, 2014) with relatively low financial cost. There are number of well-established rodent training models in the literature, e.g. swimming-induced cardiac hypertrophy model verified by echocardiography and hystomorphometry (Radovits et al., 2013) and long-term endurance training models (Benito et al., 2011; Chu et al., 2000). Nevertheless, there are numerous limitations of translating cardiac remodeling signs and mechanisms from rodents to humans, e.g. rodent hearts must contract and relax more rapidly in order to maintain cardiac output at very high heart rates (Janssen and Periasamy, 2007). Furthermore, rodent action potentials have a rapid repolarization and lack a prominent plateau phase compared to human cardiomyocytes (Nerbonne, 2004). Larger animals, such as rabbit and dog models would characterize the human heart more accurately in

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terms of oxygen uptake kinetics, cardiac mechanics, repolarization, excitation-contraction coupling, collateral coronary circulation and subcellular architecture. Considering the ion channel kinetic properties, the I_{Ks} which is presumably affected in cardiac remodeling during chronic exercise, best resembles to those measured in human hearts in dog (Liu and Antzelevitch, 1995) and rabbit ventricles (Lengyel et al., 2001). Carroll et al. used rabbits to determine the effects of exercise training during the development of obesity. They applied a 12-week-long treadmill protocol at 1.2 km·h^{-1} of maximum speed and 50-60 minute daily running sessions. Exercise trained rabbits had slower resting heart rates in both lean and obese animals (Carroll and Kyser, 2002). Such et al. found lower resting heart rates and longer ventricular effective refractory periods at all the pacing cycle lengths during *in vitro* electrical stimuli compared with the control group in a chronic motor-driven treadmill study (Such et al., 2008). Approximately 10-week endurance training programme based on treadmill running is required to get a significant cardiac response to exercise training (Hexeberg et al, 1995). Non-rodents mammals would need even more training for longer time to show the properties of athlete's heart (Wyatt et al., 1974). In our model, after a long preliminary period, 16-week-long training was used in both species at speed 2.5-3 km·h^{-1} in rabbits and at speed 6-10 km·h^{-1} in dogs, which induced lower resting heart rates, made the participating animals physically tired and sometime exhausted. As the New Zealand White rabbit is a physically inactive species, this workload is thought to be convenient to mimic regular, high-intensity human training activity. A greater similarity of rabbit myocardium to humans make them a closer representative of the human heart. The cost of acquiring and housing rabbits is still significantly much lower than for dogs.

Canine heart rate, and heart weight, excitation-contraction coupling, action potential duration and expression patterns of various ion channels are more comparable to humans (Szel et al., 2011). Moreover, canine can increase its heart rate approximately 96–136% during maximal exercise which is close-lower than the 140–170% increase in humans (Haidet et al., 1989; Musch et al., 1987; Stratton et al., 1994). These characteristics of the canine myocardium serve as a very good model of the human heart. Nevertheless, the housing and maintenance is considerably more expensive than small rodents and rabbits, the number of animals that can be used is limited, therefore, this issue is often to be taken into consideration in case of long-term studies.

5.2. Cardiac morphological changes after endurance exercise training

Physical exercise in elite athletes induces adaptation of the cardiovascular system according to the type and intensity of sport activity (Morganroth et al., 1975). Endurance trained athletes (e.g. long distance running, cross-country skiing) usually have evidence of cardiac enlargement (increased LV chamber dimensions), with or without obvious cardiac hypertrophy (increased LV wall thickness) (D'Andrea et al., 2010; Mitchell et al., 2005; Pelliccia et al., 2010; Toufan et al., 2012). The present study showed increased cardiac left ventricular end-diastolic diameters in the exercised animals. Interestingly, neither interventricular septum, nor posterior wall thickness altered. Our echocardiographic results correspond to long-term endurance exercise, indicating structural cardiac response to an increased volume load. Since the duration of long-term exercise has also an impact on the physiological remodeling of myocardial structure and function in athletes (Weiner et al., 2015), it may be possible that after several years of continuous training the morphological signs of wall thickness and ventricular hypertrophy could be seen in the model. Further studies (i.e. considerably longer training sessions) are required to verify this hypothesis.

5.3. Increased parasympathetic activity

Endurance training results in enhanced parasympathetic activity in young athletes (Jensen-Urstad et al., 1997; Macor et al., 1996), which contributes to a higher prevalence of sinus bradycardia in resting conditions and a slower increase in heart rate at any degree of submaximal oxygen uptake (Uusitalo et al., 1996) Analysis of the beat-to-beat variability of the RR intervals permits insight in this regulation mechanism in a non-invasive way. In the present study a training-induced bradycardia was found which was accompanied by an increase in HRV values in both species during drug-free resting conditions. These findings are related to a higher parasympathetic activity due to the long-term endurance training. This was supported after atropine blockade when the heart rate increased in the exercised dogs. Likewise, some studies that used heart rate variability methods, demonstrated an increase in parasympathetic activity (Goldsmith et al., 1992), and its disappearance after vagolytic maneuver. However, atropine challenge could not decrease the heart rate of exercised dogs to that level of the sedentary ones. Some studies have proposed that alterations in the intrinsic properties of the sinus node, the so-called 'non-autonomic component' was responsible for rest bradycardia of athletes (Boyett et al., 2013; Katona et al., 1982). Since we have not investigated this point at the cellular level in our study, intrinsic adaptations in the conduction system (e.g. as a result of some downregulation of I_f channel) cannot be ruled out. Considering that only moderate heart rate increment was found after atropine infusion in the exercised dog group, it is possible that long-term endurance training may also induce intrinsic adaptations in the sinus node.

5.4. Repolarization-related changes and susceptibility for cardiac arrhythmias

Several studies showed that cardiac remodelling, e.g. left ventricular hypertrophy and dilatation, induced by different pathophysiologic processes (Janse, 2004; Volders et al., 1998) prolonged repolarization, increased electrical inhomogeneity and arrhythmia propensity. However, there are less data about cardiac repolarization in exercise-induced physiological cardiac remodeling, especially in animal models. Findings from early research pointed out that repolarization BVI parameters are increased in elite soccer players (Lengyel et al., 2011), raising the possibility to an increased propensity for ventricular arrhythmias. In our study, some of the BVI QT values tended to increase in the exercised rabbits. It was hypothesised previously that endurance-exercised animals have some degree of repolarization impairment, even without clinical sign or measurable QT interval lengthening under baseline assessment. This might be unmasked by potassium channel inhibition (Lengyel et al., 2007). In this study, the selective I_{Kr} channel

blocker dofetilide tended to lengthen the QT_c intervals equally in the exercised dogs *in vivo* as compared with the sedentary group. This finding might indicate an augmented repolarization prolongation after long-term exercise in these models that could be in connection with higher risk of the repolarisation-related proarrhythmic side effects.

At a low concentration of dofetilide, which was applied in this study for the identification of mild repolarization changes, only some ventricular premature beats occurred equally in all groups. Consequently, the exercised dogs were not more susceptible to arrhythmias than the controls.

Our study did not find enough hard evidence in any investigated species about repolarization-related electrical remodeling. However, a modest repolarization alteration cannot be excluded in athletes, since the training induced repolarization changes are probably mild (alike in the human athlete's heart). Even if these effects are found to be marginal, they may be added up with other potentially harmful factors (e.g. non-steroid agents, H1 antihistamines, dietary constituents or doping) resulting in a dangerous increase of repolarization inhomogeneity forming a significant arrhythmia substrate in the athlete's heart (Varro and Baczko, 2010).

5.5. Doping

Anabolic androgenic steroid abuse has been shown to change lipoprotein metabolism leading to premature atherosclerosis, hypertension and myocardial infarction resulting in cardiomyopathy and ventricular arrhythmias (Dhar et al., 2005). However, it is unknown if testosterone or other anabolic steroid can trigger cardiac electrophysiological changes and augment proarrhythmic potential. Our results showed that testosterone in conjunction with a standardized program of endurance exercise did not increase cardiac muscle mass and did not influence other structural and repolarization parameters in dogs. Undoubtedly, some athletes and bodybuilders may use higher doses of anabolic steroids than that was used in our study. Moreover, athletes often take multiple forms of doping agents simultaneously. It may be also possible that steroid doping has different kind of effects on different type of sports. To clarify these effects of steroid doping in this model, further investigation is needed.

6. Conclusion

Long-term intensive exercise caused left ventricular cavity enlargement and changes in heart rate and in HRV parameters indicating an increased vagal tone in both species. These findings are in accordance with human cardiac remodeling in elite endurance athletes. Lower heart rate by itself may favour prolonged repolarization and inhomogeneity, furthermore, higher sensitivity to the I_{Kr} inhibitor dofetilide may explain higher risk of life-threating arrhythmias. These experimental models might be useful for the further investigations of the cardiovascular effects of the long-term physical exercise in humans.

7. Limitation

Low 'n' numbers were used in the dog studies attributed to the long training protocol and to the restrictions in access to large animals (i.e. dogs), which did not make possible the proper comparison of the groups. Similarly, the verification of mild repolarisa-

tion changes seen in top athletes, a relatively large sample size is required which is really challenging to produce with non-rodent animals. The application of higher sample size in further studies is warranted to thoroughly prove our hypotheses.

Conflict of interest

The authors declare that there is no conflict of interest.

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