Experimental and clinical investigation of short- and long-term cardiovascular effects of professional sport – Final report (2016.10.01.-2020.09.30.)

1. Experimental investigations

We investigated the reversibility of exercise-induced functional alterations in our rat model of athlete's heart. Exercised rats (DEx) completing the 12-week-long swim training program and their control littermates (DCo) remained sedentary for an 8-week-long 'detraining' period. Regular echocardiographic examinations were performed to obtain morphological parameters and speckle-tracking analysis-derived functional indices during the development and regression of exercise-induced cardiac hypertrophy. According to our echocardiographic data, a concentric left ventricular (LV) hypertrophy developed during the training program (LV mass at week 12: 0.92±0.02g DCo vs. 1.04±0.04g DEx, $p<0.01$) and showed a rapid regression after cessation of the training (LV mass at week 20: 1.02±0.02g DCo vs. 1.00±0.01g DEx, n.s.). This morphological alteration was followed by changes in speckle-tracking echocardiography derived systolic functional parameters (global circumferential strain, GCS at week 12: -14.2±0.4% DCo vs. -19.1±1.0% DEx, $p<0.01$; GCS at week 20: -12.9±0.7% DCo vs. -13.3±0.6% DEx, n.s.). Hemodynamic characterization also showed a total reversion of training-induced functional alterations: we found a complete regression of increased LV stroke volume (SV: 143.9±9.6µl DCo vs. 144.8±9.0µl DEx, n.s.), enhanced contractility (preload recruitable stroke work, PRSW: 70.9±2.4mmHg DCo vs. 69.5±2.7mmHg DEx, n.s.) and improved mechanoenergetics. LV stiffness did not differ between the groups (end-diastolic pressure-volume relationship, EDPVR: 0.029±0.004mmHg/µl DCo vs. 0.029±0.004mmHg/µl DEx, n.s.) and no myocardial fibrosis was found after regression of athlete's heart.

In additional experiments, we investigated LV diastolic function in our rat model of athlete's heart by speckle-tracking echocardiography. In the non-trained control group, early diastolic strain rate (CSrE) and isovolumic relaxation time (IVRT) indicated a significant decline in diastolic function during the 12-week-long experimental protocol (CSrE: 4.82±1.19 1/s baseline vs. 3.58±1.04 1/s 12w, $p<0.05$; IVRT: 18.4±1.9ms baseline vs. 21.9±2.2ms 12w, $p<0.001$), however, both parameters remained normal in the trained group (CSrE: 4.08±1.12 1/s baseline vs. 4.72±1.16 1/s 12w; IVRT: 19.0±2.7ms baseline vs. 19.6±2.5ms 12w; both p=NS), resulting in significant differences between exercised and control rats at the end of the training period (both $p<0.05$). In summary, in control rats, aging resulted in decreased diastolic strain rate and increased IVRT along with preserved systolic function. However, in physiological LV hypertrophy induced by exercise training diastolic strain rate and IVRT values remained normal, confirming that regular physical exercise may prevent age-related decline of diastolic function.

After providing hemodynamic characterization of athlete's heart and investigating the reversibility of exercise-induced functional alterations in our rat model of athlete's heart we provided gender-specific comparison of long-term exercise-induced alterations. We divided our young, adult male and female rats into control and exercised groups. Age-matched young adult rats were divided into female exercised (FEx), female control (FCo), male exercised (MEx) and male control (MCo) groups. Athlete's heart was induced by swim training in exercised groups (200 min/day swimming for 12 weeks). Following the training period we assessed LV hypertrophy with echocardiography, while LV pressure-volume (P-V) analysis was performed to investigate in vivo LV function. LV hypertrophy estimated by echocardiography (LV mass: +23.5% female vs. +12.3% male, $p<0.05$) and post-mortem heart weight data was more pronounced in females. Despite the more significant hypertrophy

in females, characteristic functional parameters of athletes's heart did not show notable differences between the genders during invasive hemodynamic measurements. LV P-V analysis showed increased stroke volume and stroke work, improved contractility and mechanoenergetics and unaltered LV stiffness in both males and females. Molecular biological studies were performed. The induction of Akt signaling was more significant in females (p-Akt/Akt ratio: +57.7% female vs. +21.4% male, $p<0.05$). Suppressed phosphorilation of Erk was observed in female exercised rats and we found sex-specific distinction of the mTOR-p70S6K pathway after exercise training.

Additionally, we characterized coronary artery remodeling after exercise training. The in vitro vasoconstriction, endothelium-dependent vasorelaxation, and biomechanical properties of the intramural coronary resistance arteries were investigated by pressure microarteriography. A similar outer radius and reduced inner radius resulted in an elevated wall to lumen ratio in the MEx and FEx animals compared to that in the sedentary controls. The wall elastic moduli increased in the MEx and FEx rats. Spontaneous and TxA2 agonist-induced vascular tone was increased in the FEx animals, whereas endothelium-dependent vasorelaxation became more effective in MEx rats. Coronary arteries of FEx rats showed stronger contractions, while coronaries of MEx animals showed improved dilation. The observed sport adaptation in the coronary resistance arteries of rats may contribute to a better understanding of the physiological and pathological function of these arteries in active and retired athletes of different sexes.

The cardiac ventricular electrophysiological consequences of long-term exercise training were investigated on Ca^{2+} homeostasis and ventricular repolarization, together with the underlying alterations of ion channel expression in our athlete's heart model. Animals in the trained group exhibited significantly lower resting heart rate, higher incidence of extrasystoles and spontaneous Ca^{2+} release events. The Ca^{2+} content of the sarcoplasmic reticulum (SR) and the Ca^{2+} transient amplitude were significantly larger in the trained group. Our results lead us to conclude that sudden cardiac death associated with training-induced remodeling could possibly arise as the disadvantageous consequence of Ca^{2+} homeostasis adaptation in the athlete's heart.

Based on the work plan, we established rodent in vivo intracardiac electrophysiological measurements in our laboratory and characterized atrial electrophysiological alterations after long-term exercise both in male and female animals. After exercised animals completed a 12-week-long swim training protocol, in vivo electrophysiological investigation was performed by programmed stimulation with an octapolar catheter inserted into the right atrium and right ventricle. Exercise training was associated with prolonged right atrial effective refractory period (RAERP: 43.1±4.6ms MCo, 49.8±4.2ms MEx, 40.2±5.9ms FCo, 45.7±4.3ms FEx, $p_{ex}<0.01$) and unaltered sinus node recovery time (SNRT), while we could not induce significant number of arrhytmias by programmed stimulation (double extrastimulation, burst pacing) in exercised groups. We found decreased expression of potassium channels participating in atrial repolarization and connexin-43. Female gender was associated with lower profibrotic expression and collagen density. Myocardial hypertrophy was verified by post-mortem atrial and total heart weight data in both exercised groups. We found increased atrial gene expression of antioxidant enzymes (e.g. NADPH oxidase 2, superoxide dismutase 2) in both genders, that might be the consequence of transient oxidative stress during training sessions. Histological evaluation confirmed atrial hypertrophy in male and female exercised rats compared to controls. Despite the marked atrial hypertrophy, no gene expression alteration was found regarding markers that describe pathological remodeling (atrial natriuretic factor, β-myosin heavy chain), proinflammatoric (tumor necrosis factor-α) and profibrotic [e.g. transforming growth factor-β (TGF-β), matrix metalloproteinase-2 (MMP-2)] processes. This was underlied with unaltered collagen content of atrial myocardium (right

atrial collagen area percentage: 10.4±1.2% MCo, 9.1±0.9% MEx, 7.7±0.8% FCo, 6.9±0.7% FEx). The unaltered Bax/Bcl-2 ratio suggested unchanged apoptotic activity. While exercise training did not effect on the expression of profibrotic markers, female sex was associated with lower TGF-β and MMP-2 expression.

The planned studies for the examination of „exercise-induced cardiac fatigue" have been performed. Rats of the exercise group were forced to swim for 3h with 5% body weight (workload) attached to the tail, control rats were taken into the water for 5min. After 2, 24 and 72h recovery periods we performed echocardiographical follow-up and left ventricular (LV) pressure-volume analysis to investigate LV function and mechanoenergetics.

We observed increased end-systolic volume, decreased ejection fraction (44±3 vs 60±2%), impaired contractility (preload recruitable stroke work) and mechanoenergetics (ventriculoarterial coupling, mechanical efficiency) of LV along with parallel troponin positivity in the exercised group after 2h follow-up. These functional changes tended to be reversible at 72h. After completion of these experimental series we plan to publish our results in the next year.

2. **Clinical investigations**

We started our research project in the first year with the inclusion of 25 elite adult athletes (mainly waterpolo players and swimmers, most of them are members of the Olympic team), 80 non-elite junior athletes (mainly football players) and 15 master athletes (mainly long-distance runners and waterpolo players) in our clinical investigations. The athletes underwent the evaluation of anamnestic data by a detailed questionnaire. We also created a new anonymus questionnare about what the athletes think and know about health, prevention and screening. Up to the present, we already collected the first 200 filled questionnaires. We implemented the routine physical examination of the athletes with body composition analysis. Blood pressure measurement, 12-lead ECG analysis, and two-dimensional and also three-dimensional echocardiography were carried out routinely. Every elite athletes and non-elite junior athletes underwent cardiopulmonary exercise testing with lactate measurements in every 2 minutes, every master athletes had routine treadmill exercise ECG examinations. Cardiac magnetic resonance (CMR) examinations were performed in 84 athletes. Cine images were performed in long-axis, RVOT, LVOT and short-axis views, velocity-encoded phase contrast flow measurements in the aorta and pulmonary trunk. In 71 athletes routine laboratory biochemical tests (including high-sensitive Troponin T) were carried out and we also collected and conserved blood samples for further experiments (such as RNA isolation). Parallelly with the above mentioned clinical examinations, we have started the creation of complex databases and filling up our data into them. Processing and analysis of our first data as well as the statistical work have already been started. Detailed echocardiographic and cardiopulmonary exercise testing post-processing was completed in every investigated athlete and the results were included in our database. CMR post-processing was also completed, left and right vetricular volumes, ejection fractions and masses and additional derived parameters were evaluated. Nine medical students were also involved in our clinical examinations and scientific work. Our first results proved the high prevalence of cardiovascular risk factors and cardiovascular abnormalities in master athletes, revealed the importance of cardiovascular screening in young athletes as well, and indicated the need for specific education programmes for athletes and trainers in the field of sports activity and health.

In the second year, we included further 25 elite adult athletes (mainly kayak and canoe athletes and swimmers, most of them are members of the Olympic team), 120 non-elite junior athletes (mainly waterpolo and football players, swimmers and fencers), 15 master athletes (mainly long-distance runners and waterpolo players), 50 NB1 handball referees and 40 control nonathlete person (young and adult) in our human clinical investigations. We

collected further 200 anonymus questionnare about what the athletes think and know about health, prevention and screening. We also created a similar questionnaire for referees, and we collected the first 50 filled referee questionnaires. Detailed follow-up of 30 elite waterpolo players and 20 junior football players was also carried out. We have started to analyse the gender differences of young and adult elite athletes as well applying our multiple investigational modalities. Parallelly with the above mentioned clinical examinations, processing and analysis of our data and the statistical work is ongoing, the creation of our complex database is under completion. Nine medical students were also involved in our clinical examinations and scientific work. Our follow up results proved the applicability and usefulness of the stress physiological examinations we have set up at the beginning of the the study in adults and young athletes as well. Our results of handball referee screening proved the importance of cardiovascular screening of referees as well, undergoing similar physical and psychological stress in match situations as the athletes. As we saw the need for specific education programmes for referees in the field of sports activity and health, we also gave scientific lectures about the first results of the screening to them. In the second year, a total of 60 elite water polo athletes (19±4 years, 17±6 hours of training/week, 50% female) and 40 healthy, sedentary controls were enrolled in the echocardiographic examinations. We measured RV end-diastolic volume index (RVEDVi) and ejection fraction (RVEF) using dedicated software. Furthermore, we have determined RV global longitudinal (RV GLS) and circumferential strain (RV GCS) and the relative contribution of longitudinal (LEF) and radial ejection fraction (REF) to RVEF using the ReVISION method. Athletes also underwent cardiopulmonary exercise testing (VO_2/kg). Athletes had significantly higher RVEDVi compared to controls (athletes vs. controls; 88±11 vs. 65±10ml/m^2, $p<0.001$), however, they also demonstrated lower RVEF (56±4 vs. 61±5%, $p<0.001$). RV GLS was comparable between the two groups (-22±5 vs. -23±5%, $p=0.24$), while RV GCS was significantly lower in athletes (-21±4 vs. -26±7%, $p<0.001$). Athletes had higher longitudinal and lower radial contribution to RVEF (LEF/RVEF: 0.50±0.07 vs. 0.42±0.07, $p<0.001$; REF/RVEF: 0.33±0.08 vs. 0.45±0.08, $p<0.001$). Moreover, the pattern of RV functional shift correlated with VO_2/kg (LEF/RVEF: $r=0.30$, $p<0.05$; REF/RVEF: $r=-0.27$, $p<0.05$). In conclusion, RV mechanical adaptation to long term, intense exercise imply a functional shift: the relative contribution of longitudinal motion to global function is increased, while the radial shortening is significantly decreased in athletes. Moreover, this functional pattern correlated with aerobic exercise performance, representing a potential new resting marker of athlete's heart. We also aimed to characterize female athlete's heart in elite competitors in the International Federation of Bodybuilding and Fitness (IFBB) Bikini Fitness category and compare them to athletes of a more dynamic sport discipline and healthy, sedentary volunteers using 3D echocardiography. Fifteen elite female fitness athletes were recruited and compared to 15 elite, age-matched female water polo athletes and 15 age-matched healthy, nontrained controls. Left ventricular mass index was significantly higher in the athlete groups; the hypertrophy, however, was even more prominent in water polo athletes (78±13 versus 91±10 versus 57±10g/m^2, $p<0.0001$). To the best of our knowledge, this is the first study to characterize female athlete's heart of IFBB Bikini Fitness competitors. The predominantly static exercise regime induced a mild, concentric-type LV hypertrophy, while in water polo athletes higher ventricular volumes and eccentric LV hypertrophy developed.

We continued our studies in the third year with the inclusion of further 50 elite adult athletes (mainly waterpolo players and swimmers, most of them are members of the Olympic team), 60 elite junior athletes (mainly elite waterpolo players and swimmers), 15 master athletes (mainly long-distance runners and waterpolo players), 50 NB1 handball referees and 20 control nonathlete person (young and adult). We collected further 50 anonymus questionnares from handball referees about health, prevention and screening. Detailed follow-

up of 50 elite waterpolo players and swimmers was also carried out. On the basis of the detailed follow-up of elite adult athletes, we processed the technique of systematic evaluation and application of stress physiology parameters in training planning together with the trainers and team doctors. We supplemented the routine screening of elite athletes with the measurement of ventricular repolarisation heterogeneity as a potential tool for characterization of electrical remodeling of the athlete's heart. Parallely with the above mentioned clinical examinations, processing and analysis of our data, the statistical work and the clinical testing of our complex database is ongoing. Sixteen medical students were also involved in our clinical examinations and scientific work. We continued the analysis of the gender differences in young and adult elite athletes applying our multiple investigational modalities. We started to investigate the influence of age, training hours per week and the total number of years of training on the signs of cardiovascular sport adaptation. Moreover, the analysis of the changes of cardiac markers in the blood samples of athletes before and after training has also been started. The growing number of our control measurements provided the ground for systematic anatomical and functional comparison between non-athlete and athlete hearts.

The results of junior athlete examinations proved the importance of early cardiovascular screening and follow-up: treatment for hypertension, iron deficiency or different kinds of arrhythmias was applied in 8% of the cases, while regular follow-up for anatomical or electrophysiological abnormalities, cardiovascular risk factors or for factors limiting performance was suggested in near 14%. Master athlete screening showed the high number of athletes requiring cardiovascular treatment: antihypertensive treatment was initiated in 12% lipid lowering treatment in 8% of them, while the restriction of sport activity was recommended in 10 %, due to ischaemic heart disease in most of the cases.

In our echocardiography studies, we aimed at the characterization of left atrial (LA) and left ventricular (LV) remodeling using 3D echocardiography in elite athletes and their correlation with exercise capacity. Via a retrospective analysis, the current study group consisted of 138 elite athletes (mean age, 20±4 years; 62% men) and 50 sedentary control subjects. Electrocardiographically gated full-volume 3D datasets were obtained for offline analysis using dedicated software for 3D LA and LV measurements. Body surface area–indexed LA maximal volume (LAV_{max}) and LVEDV were determined. LA total emptying fraction, LA passive and LA active emptying fraction, and LV global longitudinal strain (GLS) were also calculated. Athletes also underwent cardiopulmonary exercise testing to determine peak oxygen uptake. Athletes demonstrated higher 3D LAV_{max} (32±6 vs 26±8ml/m^2) and indexed LVEDV (85±12 vs 62±10ml/m^2) compared with control subjects ($P<0.001$ for both). Functional measures of the left ventricle and left atrium, such as the absolute value of 3D LV GLS (19±2 vs 22±2%), LA total emptying fraction (58±6 vs 64±6%), and active emptying fraction (24±10 vs 32±10%) were lower in athletes ($P<0.001$ for all). Male athletes had higher indexed LVEDV compared with female athletes (89±13 vs 80±8ml/m^2, $P<0.001$), but LAV_{max} did not differ between genders (32±6 vs 33±5ml/m^2 , $P=0.18$). Besides heart rate, gender, and body surface area, 3D LAV_{max}, LV GLS, and LA passive emptying fraction were independent predictors of peak oxygen uptake. In conclusion, we have found that regular physical exercise results in marked LA and LV remodeling with considerable gender differences as explored by 3D echocardiography. In contrast with various cardiovascular diseases, more pronounced LA dilation and lower resting functional measures are associated with better exercise performance.

Furthermore we have summarized all of the athletes who were examined using cardiac magnetic resonance (CMR) imaging. A total of 327 athletes (242 male) were included (adults>18y; adolescents 14–18y). Athletes were categorised as skill, power, mixed and endurance athletes. Male athletes had higher LV and RV volumes and masses in both adult

(n=215 (145 male); 24±5y) and adolescent (n=12 (97 male); 16±1y) groups compared with women (all $p<0.05$). In adults, male sex, age, body surface area, weekly training hours, mixed and endurance sports correlated with higher ventricular volumes and masses (all $p<0.05$); and a combination of age, sex, training hours, endurance and mixed sports explained 30% of the variance of the left ventricular end-diastolic volume index (r=0.30), right ventricular end-diastolic volume index (r=0.34), right ventricular mass index (r=0.30); and as much as 53% of the left ventricular mass index (r=0.53)(all $p<0.0001$). In adolescents, positive correlations were found between training hours and left ventricular hypertrophy (r=0.39, $p<0.0001$), and biventricular dilation (left ventricular end-diastolic volume r=0.34, p=0.0008; right ventricular end-diastolic volume r=0.36, P=0.0004). In adolescents, age and body surface area did not correlate with CMR parameters.

Our results regarding the role of CMR in the differentiation of athlete's heart and hypertrophic (HCM) or arrhythmogenic right ventricular cardiomyopathy (ARVC) were successfully published. We included 194 non-athlete HCM patients (50.2±13.6y, 108 male), 150 highly trained healthy athletes (24.2±4.8y, 101 male) and ten additional athletes with HCM (31±10y; 9 male, 14.4±6.5 training hours/week). Cut-off value for ratio of $EDWT/LVEDVi_{CQ}$ less than 0.14 $mm \times m^2/ml$ and cut-off value for $EDWT/LVEDVi_{TQ}$ less than 0.17 discriminated between physiological and pathological LV hypertrophy with a sensitivity of 99.5% and 99.0%, a specificity of 98% and 99.3%, respectively. Cut-off value for ratio of $LVM/LVEDV_{CQ}$ less than 0.82 $mm \times m^2/ml$ and cut-off value for $LVM/LVEDV_{TQ}$ less than 1.27 discriminated between physiological and pathological LV hypertrophy with a sensitivity of 77.8% and 89.2%, a specificity of 86.7% and 91.3%, respectively. According to our cut-off values $EDWT/LVEDVi_{CQ}$, $EDWT/LVEDVi_{TQ}$, $LVM_{CQ}/LVEDVi_{CQ}$ and $LVM_{TQ}/LVEDVi_{TQ}$ were in the pathological range in 9, 9, 8 and 10 athletes with HCM, respectively.

In our study regarding CMR characteristics of healthy athletes (HA), sedentary HCM and athletic HCM patients we aimed to determine CMR parameters which can help to diagnose HCM in athletes.We included male sedentary HCM patients with slightly elevated maximal end-diastolic wall thickness (EDWT 13-18 mm, n=40, 47.6±14.7y), HA (n=30, 27.5±5.6y) and athletes with HCM (n=16, 29.6±13.4 y). We determined conventional and derived parameters such as EDWT/LVEDVi, LVM/LVEDV ratio and strain parameters such as global longitudinal (GLS), radial (GRS) and circumferential strain (GCS), SD of peak LS and CS using feature tracking. The univariate regression model showed that LVEF, EDWT, EDWT/LVMi, LVM/LVEDV, GCS, GRS, SD of peak LS and CS are determinants of the diagnosis of HCM among athletes. Multivariate regression revealed that EDWT/LVMi and GCS are independent disease predictors in athletes ($p<0.05$). Cut-off value for GCS $\leq$-32.5 and for EDWT/LVEDVi >0.126 discriminate athletic HCM from HA with a sensitivity of 81.3 and 87.5% (AUC 0.93), and a specificity of 96.7 and 83.3% (AUC 0.95), respectively.

In the other study 34 non-athlete ARVC patients (40.5±13.4 years, 22 male), 34 healthy athletes and 8 additional athletes with ARVC (18.9±4.6 training h/week) were enrolled. Establishing AUC values for the CMR parameters RVEF (cut-off: $\leq$45.8) showed good accuracy (AUC=0.830), whereas RVEDVi failed as a discriminator between ARVC and athlete's heart (AUC=0.599). Cut off values for RV mid strain (>-25.6), average (>-29.4) and minimum of the measured regional strain values (>-18.1) demonstrated good discrimination between athlete's heart and ARVC. Applying the established cut-off values, RVEF was in the pathological range in only 3 athletes with ARVC. Half of the athletes with ARVC showed normal RV GLS. Regional longitudinal strain and strain rate of the RV mid free wall were in the pathological range in all 8 athletes with ARVC.

In the last year of our research project, the follow up of the athletes involved in the first three years was carried out. Moreover, we included further 30 elite adult athletes (mainly

wrestlers, members of the adult national team), and 80 elite junior athletes (mainly elite waterpolo players and handball players, most of them were members of the age matched national teams).

Processing and analysis of our data and the statistical work was completed. Detailed ECG, echocardiographic, cardiopulmonary exercise testing and CMR post-processing was completed in every investigated athlete and the results were included in our database. In particular, our echocardiographic database includes 425 athletes with complete 2D and 3D image acquisitions and analysis. This database will be used to derive the normative values used in the everyday clinical practice and it is the basis for further advanced analytical projects (i.e. by machine learning). Our projects that were completed in the last year of the research were as follows:

In a retrospective analysis, we measured resting serum levels of hsTroponinT, CKMB, LDH and NT-proBNP in 237 athletes (male:144, age:19.1±5.9 years, training:16.0±6.7 hours/week) and 53 non-athlete controls (male:23, age:19.8±3.2 years). In athletes, increased resting cardiac marker levels were measured as follows: CKMB 6.3% (n=15), LDH 3.4% (n=8), hsTroponinT 4.2% (n=10), NT-proBNP 0.8% (n=2) of the cases. No elevation of CKMB and hsTroponin T levels were measured in the control group, while only single cases of increased LDH and NT-proBNP were detected. We measured higher levels of CKMB (17.6±7.3 vs 12.3±3.4 U/l, p<0,001), LDH (322.4±60.8 vs 286.0±51.1U/l, p<0.001) and hsTroponinT (6.2±4.7 vs 4.3±1.4ng/l, p<0.05), while lower levels of NT-proBNP (23.9±27.2 vs 49.8±38.7pg/ml, p<0.001) in athletes compared to the control group. In male athletes, higher levels of CKMB (18.5±6.6 vs 16.0±8.2U/l, p<0.001), LDH (337.0±62.2 vs 300.7±51.9U/l, p<0.001) and hsTroponinT (7.0±5.3 vs 4.3±1.9ng/l, p<0.001), and lower levels of NT-proBNP (19.8±23.1 vs 35.0±34.1pg/ml, p<0.001) were measured compared to female athletes. Levels of hsTroponinT decreased in athletes due to increasing age (r=-0.20, p<0.05). According to our results, resting levels of cardiac markers showed significant alterations due to sport adaptation of the heart. These changes depended on age and sex as well.

We also carried out the complex medical screening and detailed sports cardiology assessment of the Hungarian national adult and junior swimming team. All athletes underwent detailed sports cardiology screening which consisted of medical history, resting ECG, blood test, body composition analysis, echocardiography and cardiopulmonary exercise testing (CPET). Additional examinations (ABPM, Holter), consultation with other specialists were carried out if needed. The screening was performed on 64 professional swimmers (adults: n=33, age=24.1±3.8, male: 55%, junior: age=16-9±1.2, male: 45%). The blood test identified iron deficiency of 41(64%) swimmers (adult: n=14). The juniors had lower ferritin (FE) level than the adults (59.1±32.5 vs. 98.5±57.0µg/l; p<0.01), female swimmers had lower FE level than males. 24 (38%) athletes had vitamin D deficiency. There was no pathological disorder on the resting ECG. ABPM was performed in 6 cases due to high blood pressure. On the CPET there was no difference between the performance of adult and junior swimmers. Males had higher aerobic capacity (58.8±5.1 vs. 52.2±4.4ml/kg/min; p<0.0001) and ventilation (159.1±27.3 vs. 115.8±16.1l/min; p<0.0001) than females. Peak VO_2 showed positive correlation with FE level. Pulmonology examination was needed for 13 (20%) swimmers, in 10 cases the former asthma therapy was modified, 2 athletes were newly diagnosed with asthma. Gynecology examination was needed in 9 cases. The complex medical screening of the national swimming team identified many performance-limiting disorders, which were treated. The athletes' results reflect the success of our examination: World Record and a junior championship record were set in this year.

To characterize electrical alterations in the athlete's heart, we analyzed 1 minute resting ECG I recordings (WIWE mobile ECG system) of 149 healthy young and adult elite

athletes (age: 20.7±4.8 years, male: 58%, training: 20.3±5.4 hours/week) and 92 non-athletic controls (age: 22.0±5.1, male: 59%). In addition to standard ECG parameters, certain time domain metrics of heart rate variability (AVNN, SDNN, RMSSD), mean QRST integral and relative standard deviation of QRST integrals were determined. In athletes, higher P-wave amplitude (0.9±0.3 vs. 0.8±0.2mm, $p<0.01$), PQ interval (150.5±20.4 vs. 143.7±28.3ms, $p<0.05$), T-wave amplitude (2.5±0.9 vs. 1.9±0.7mm, $p<0.01$), QRS width (92.3±12.4 vs. 85.0±11.3ms, $p<0.01$) and QTc duration (398.1±22.1 vs. 385.9±24.7ms, $p<0.01$) were measured compared to controls. Regarding resting heart rate variability, athletes had higher AVNN (850.2±155.6 vs. 786.2±121.9ms, $p<0.01$), SDNN (65.6±29.2 vs. 55.1±22.5ms, $p<0.01$) and RMSSD (52.4±33.8 vs. 43.4±21.8ms, $p<0.05$) values than controls. Mean QRST integral was higher in athletes than controls (36.0±12.6 vs. 27.2±11.1mV*ms, $p<0.01$), and also in male athletes than females (40.9±12.9 vs. 32.3±11.1mV*ms, $p<0.01$). Relative standard deviation of QRST integrals did not differ between the groups. According to our results, short-term resting ECG measurements proved to be suitable for characterizing electrical remodeling in the athlete's heart by detecting standard ECG, heart rate variability and QRST integral changes. Although detecting electrical changes, our measurements did not confirm an increased ventricular arrhythmia risk in healthy top athletes, may indicate a risk reduction instead.

We also set up the methods of field measurements (continuous heart rate monitorisation, regular lactate measurements and blood pressure measurements) and carried out the first examinations in 19 adult and 16 young elite waterpolo players. Comparing the results of laboratory and field measurements, our first results proved the usefulness of combining these techniques in the follow up of physical fitness, cardiovascular adaptation and training planning.

We also performed field testing measurements on swimmers and football players. Our aim was to create and test sport-specific field testing methods and compare their value to cardiopulmonary exercise (CPX) testing. During the exercise physiology laboratory exam, in the frame of our detailed sports cardiology screening, we performed CPX tests with 2-minutes lactate measurements. Exercise physiology field testing was performed on the soccer field (Yoyo test) and in the swimming pool with heart rate and lactate measurements. We included a young soccer team (n=17, 15.6±0.5years) and national swimmers (n=10, 23.2±5.3years). On CPX test, soccers and swimmers had excellent performance (16.9±0.9 vs. 15.4±1.4MET), aerobic capacity (59.1±2.9 vs. 54.0±4.8ml/kg/min) and ventilation (147.6±17.5 vs. 152.1±23.8l/min). In soccer players, treadmill performance was correlated with field testing performance, peak lactate (10.9±1.7 vs. 11.1±3.2mmol/l, r~0) and peak heart rate (198±6 vs. 196±6/min, r=0,7) was similar as the maximal results on the field testing. In swimmers, treadmill performance did not correlate with the competition-related endurance test performance, peak lactate was higher (9.9±3.6mmol/l vs. 12.4±2.0, r~0), peak heart rate was lower (189±10/min vs. 158±17/min, r=-0,8). Combined laboratory and field testing is an effective method of athletic performance evaluation. We created field testing methods which are easy to perform, evaluate and reproduce. In swimmers CPX tests results can help to prescribe dryland training programs, field tests are essential for design competition situation and evaluate swimming performance accurately.

In our study regarding to pathological hypertrophy, we examined the electrocardiographic predictors of left ventricular hypertrophy and myocardial fibrosis in HCM. We investigated 146 patients with HCM and 35 healthy individuals who underwent CMR with late gadolinium enhancement and standard 12-lead ECGs. The sensitivity of the Romhilt-Estes score was the highest (75%), and this hypertrophy criterion had the strongest correlation with the LVM index ($p<0.0001$; $r=0.41$). The amount of fibrosis was negatively correlated with the Cornell index ($p=0.015$; $r=-0.201$) and with the Sokolow-Lyon index

(p=0.005; r=-0.23), and the Romhilt-Estes score was independent of fibrosis (p=0.757; r=0.026). Fragmented QRS and strain pattern predicted more fibrosis, while the Cornell index was a negative predictor of myocardial fibrosis (p<0.0001). Among others, the strain pattern was an independent predictor of the LVM (p<0.0001).

In another study we investigated the prognostic significance of CMR-based markers in 187 patients with HCM. The combined endpoint of our study was all-cause mortality, heart transplantation, malignant ventricular arrhythmias and appropriate implantable cardioverter defibrillator (ICD) therapy. The arrhythmia endpoint was malignant ventricular arrhythmias and appropriate ICD therapy. The LVMi was an independent CMR predictor of the combined endpoint independent of the quantification method (p<0.01). The univariate predictors of the combined endpoint were LVMi, global longitudinal (GLS) and radial strain and longitudinal MD (MDL). The univariate predictors of arrhythmia events included LVMi and myocardial fibrosis. More pronounced LV hypertrophy was associated with impaired GLS and increased MDL. More extended myocardial fibrosis correlated with impaired GLS (p<0.001).

In the last decade, numerous studies have proven the sensitivity and the prognostic value of analyzing myocardial deformation, specifically measuring global longitudinal strain (GLS). However, GLS is largely dependent on the afterload and the morphology of the left ventricle (LV). In athletes, this often results in a decrease in GLS without pathological processes, making differential diagnosis challenging. By indexing strain to the afterload and by the detailed analysis of the pressure-strain loop, myocardial work offers a new prospect, excluding the aforementioned limitations. We enrolled 30 competitive swimmers (19±4 years, 50% male, 23±5 training hours/week) and 23 sedentary healthy volunteers adjusted for age and gender. We measured LV GLS by 2D speckle tracking and calculated myocardial work using dedicated software (GE AFI). We have quantified global work index (GWI: the area under the pressure-strain loop), global constructive work (GCW: work by shortening during systole and lengthening during isovolumic relaxation) and global wasted work (GWW: work by lengthening during systole and shortening during isovolumic relaxation). In athletes, GLS was significantly decreased compared to controls (-17.6±1.9 vs. -18.7±1.3%, p<0.05), meanwhile GWI and GWW were similar (GWI: 1850±299 vs. 1755±189Hgmm%, p=NS; GWW: 76±40 vs. 61±42Hgmm%, p=NS). Importantly, GCW was supernormal in athletes pointing at the increased efficiency of athlete's heart (2097±293 vs. 1943±213Hgmm%, p<0.05) even during resting conditions. According to our results, myocardial work analysis may be free of known limitations concerning strain values and therefore, may better characterize athlete's heart and may help to better diagnose underlying pathological processes.