Appendix

Physical examination and blood samples

A physical examination and blood samples are taken to evaluate cardiovascular risk factors (hypertension, dyslipidaemia, diabetes, smoking, and body mass index).

12-lead ECG

A resting 12-lead ECG will be recorded and interpreted in accordance to the Seattle Criteria by a cardiologist with experience in sports medicine.[1]

Dual-X-ray absorptiometry

Subjects will undergo a dual-energy X-ray absorptiometry scan (Discovery W, Hologic Inc, Bedford, Massachusetts, USA - GE Lunar Prodigy Advance, GE Healthcare, Horton, Norway) to measure lean mass, fat mass, bone mineral content and bone mineral density of the whole body, trunk, legs and arms.

Exercise testing

Peak oxygen consumption (peak VO_2) will be determined in a sports-specific manner when possible. Cyclist and triathletes will perform a continuous bicycle stress test using either a dedicated ergometer (Lode Excalibur, Lode, Netherlands) or with the athlete's racing bicycle attached to a cycle ergometer (Avantronic Cyclus II, Leipzig, Germany). As we do not have the infrastructure to test swimmer, rowers and cross-country skiers in a sport-specific manner these athletes will also perform an bicycle exercise test. The protocol is determined at each site with either a ramp or incremental step of increased workload until exhaustion after a brief warm-up at low power. Runners performed a running test on an electronically controlled treadmill (Venus®

200/75, H/P/cosmos, Nussdorf, Germany). After a 5-minute warm-up, the starting will be 8 $km.h^{-1}$ and will increased by 1.5$km.h^{-1}$ every 8-min until exhaustion. Respiratory gas exchange will be analysed using a breath-by-breath open circuit spirometry system (Vyntus CPX Metabolic Cart, Vyaire Medical, Germany). Peak VO_2 will be determined as the highest 30s average oxygen consumption. The first and second ventilatory threshold will be determined from respiratory gas analysis parameters. During this test, a continuous 12-lead ECG will be recorded at a speed of 25 mm/s. The exercise ECG will be interpreted for repolarization abnormalities and arrhythmias by a cardiologist with experience in sports medicine.

Two- and three-dimensional transthoracic echocardiography

Two- and three-dimensional TTE will be performed using a Vivid E9 or E95 ultrasound system (GE Healthcare, Horton, Norway) with an active matrix single-crystal phased array transducer (GE M5Sc-D probe, GE Healthcare, Horton, Norway) and 1.5-4MHz matrix-array transducer (GE 4Vc-D Matrix 4D cardiac probe, GE Healthcare, Horton, Norway). Cardiac morphology will be assessed, including end-diastolic (EDV), end-systolic volumes (ESV), rendering ejection fraction (EF) for both ventricles, as well as right and left atrial volumes. Diastolic function will be assessed using established Doppler and tissue-Doppler parameters such as the E wave velocity, the A wave velocity, the E/A ratio, septal, lateral and averaged E', E/E', tricuspid regurgitation flow velocity and the S-D-A waves at the pulmonary veins. In depth analysis of the intrinsic myocardial function will be performed by strain analyses. RV and LV strain and strain rate will be assessed as measures of systolic function. RV and LV early and late diastolic strain rate will be assessed for diastolic function. Time-to-peak shortening in all 18 segments of the LV and of the RV free wall will be measured to assess for differences in timing and mechanical dispersion. Atrial strain analysis will

be performed to assess the reservoir, conduit and contraction function of both atria. All measurements will be made following international guidelines. [2 3]

Cardiac Magnetic Resonance Imaging

Cardiac magnetic resonance imaging will be performed using a 1.5T MRI scanner (Magnetom Aera and Sigma 1.5T – Siemens Healthineers, Erlangen, Germany; Ingenia, Achieva or Ambition 1.5T - Philips Medical Systems, Best, The Netherlands) or a 3.0T scanner (Prisma, Siemens Healthineers, Erlangen, Germany) using a dedicated cardiac coil and electrocardiographic gating. Steady-state free precision (SSFP) short-axis cine imaging (8 mm slice thickness without gaps) will be obtained to analyse cardiac mass, function and volumes. In addition, native and post-contrast T1 mapping will be performed using the modified look-locker inversion recovery (MOLLI) sequence to calculate extracellular volume (ECV). Myocardial fibrosis (MF) will also be evaluated by means of delayed enhancement on breath hold phase-sensitive inversion recovery (PSIR) sequences 10 minutes after administration of gadolinium-DTPA. Analysis of CMR data will be performed in a central core lab. Assessment of cardiac volumes and mass will be performed using RightVol (KU Leuven, Leuven, Belgium). IntelliSpace Portal (Philips Medical Systems, Eindhoven, The Netherlands) will be used for T1 and ECV mapping and Suiteheart (Neosoft, Pewaukee, USA) is used for strain analysis (feature tracking). Our validated robust non-rigid motion correction will be used for accurate T1 measurements and ECV calculations.[4]

Exercise Cardiac Magnetic Resonance Imaging

In the University Hospital of Leuven and at the Baker Heart and Diabetes Institute Melbourne an exercise CMR will be performed using a cycle ergometer with adjustable electronic resistance (Lode, Groningen, The Netherlands). Images will be acquired using a Philips Achieva 1.5 T CMR

with dedicated cardiac coil during supine bicycle exercise at 25%, 50%, and 66% of maximal power determined by previous upright cardiopulmonary exercise testing. Steady-state free precession cine imaging was performed without cardiac gating. A 3D stack of 13–18 contiguous 8 mm image slices, covering both ventricles from apex to base, will be serially acquired in the short-axis plane and the horizontal long-axis plane. All image frames will be acquired during free breathing. Ventricular and atrial volumes and functional reserve during exercise will be measured using RightVol (KU Leuven, Leuven, Belgium). Our research group has previously demonstrated the feasibility, reliability and clinical utility of CMR to quantify cardiac volumes and function during exercise. [5 6]

Exercise stress echocardiography

In the University Hospital Antwerp and Jessa Hospital Hasselt, exercise stress echocardiography (Vivid E95 ultrasound system - GE Healthcare, Horton, Norway) will be performed instead of exercise CMR in the subjects having undergone CMR at rest. An exercise table (ER900 and Oxycon Alpha, Jaeger, Germany) allowing backward and side tilt for optimal signal sampling will be used. Measurements will be performed at rest and during several stages of exercise depending on heart rate and respiratory gas analysis parameters. The following measurements and derived calculations will be collected: LVEF, RVEF, RV fractional area change, biventricular systolic and diastolic strain parameters, Doppler and tissue Doppler parameters to assess cardiac output, diastolic function and pulmonary artery systolic pressure. Using stress echocardiography we will assess how prolonged high intensity endurance training impacts pulmonary vascular resistance and diastolic function during exercise. All measurements will be made following international guidelines. [7]

Holter analysis

For the 24-hour Holter ECG monitoring, a Spiderview Holter device (Ela Medical, Paris, France) or a Pocket ECG (Device Solution, Belrose, New South Wales, Australia) will be attached to BlueSensor VL ECG electrodes (AmbuR, Penang, Malaysia). The ECG recordings will be analysed offline using SyneScope software (ELA Medical, Paris, France) to determine heart rate extremes, heart rate variability and the prevalence of arrhythmias.

Electronic Training Diary

Physiological data obtained with heart rate monitors during training sessions will enable quantification of exercise intensity during representative training bouts. Online tracking of sports activity will be performed using a dedicated web-based platform compatible with all current heart rate monitor and sports GPS devices (TrainingPeaks®, Peaksware, Boulder, USA). [8 9] All athletes will receive a user profile upon study enrolment and permission will be asked to track all sports activities using a central coach profile. The recorded data on duration, distance, altitude, speed, power output and heart rate of from training sessions will be exported and transformed using a big data bioinformatics platform (RStudio 2020, Integrated Development for R, PBC, Boston, MA, USA). Finally, training load will be expressed by a parameter combining a factor of duration and intensity (e.g. Banister training impulse [TRIMP] and derivatives) will be further quantified for any relevant time period (week, month and year).[10 11]

Genetic testing

Our primary genetic analyses will focus on (i) rare variants in cardiomyopathy associated genes, and (ii) polygenic risk scores (PRS) for various cardiac traits including DCM and AF.

Rare variants will be detected using a custom gene array comprised of protein-coding sequences of 24 genes that have been curated to show strong evidence of association with DCM, HCM or ARVC. Sequencing data will be analysed using an in-house pipeline at the VCCRI. Variant pathogenicity will be assessed according to standard clinical guidelines using the American College of Medical Genetics and Genomics scoring matrix. [12]

Genome-wide evaluation of single nucleotide polymorphisms will be obtained using the Axiom Precision Medicine Diversity Array (PMDA). Data will be used to derive PRS for various cardiac traits using validated scoring algorithms.

Finally, extracted DNA will be stored for potential future whole-genome sequencing.

1. Sharma S, Drezner JA, Baggish A, et al. International recommendations for electrocardiographic interpretation in athletes. *European Heart Journal* 2018;39(16):1466-+. doi: 10.1093/eurheartj/ehw631
2. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging* 2015;16(3):233-70. doi: 10.1093/ehjci/jev014 [published Online First: 2015/02/26]
3. Nagueh SF, Smiseth OA, Appleton CP, et al. Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging* 2016;17(12):1321-60. doi: 10.1093/ehjci/jew082 [published Online First: 2016/07/17]
4. Tilborghs S, Dresselaers T, Claus P, et al. Robust motion correction for cardiac T1 and ECV mapping using a T1 relaxation model approach. *Med Image Anal* 2019;52:212-27. doi: 10.1016/j.media.2018.12.004 [published Online First: 2019/01/01]
5. La Gerche A, Claessen G, Van de Bruaene A, et al. Cardiac MRI: a new gold standard for ventricular volume quantification during high-intensity exercise. *Circ Cardiovasc Imaging* 2013;6(2):329-38. doi: 10.1161/CIRCIMAGING.112.980037 [published Online First: 2012/12/22]
6. Claessen G, Schnell F, Bogaert J, et al. Exercise cardiac magnetic resonance to differentiate athlete's heart from structural heart disease. *Eur Heart J Cardiovasc Imaging* 2018;19(9):1062-70. doi: 10.1093/ehjci/jey050 [published Online First: 2018/03/29]
7. Lancellotti P, Pellikka PA, Budts W, et al. The clinical use of stress echocardiography in non-ischaemic heart disease: recommendations from the European Association of Cardiovascular Imaging and the American Society of Echocardiography. *Eur Heart J Cardiovasc Imaging* 2016;17(11):1191-229. doi: 10.1093/ehjci/jew190 [published Online First: 2016/11/24]

8. Wallett AM, Woods AL, Versey N, et al. Effect of Intensified Endurance Training on Pacing and Performance in 4000-m Cycling Time Trials. *Int J Sports Physiol Perform* 2018;13(6):735-41. doi: 10.1123/ijspp.2017-0287 [published Online First: 2017/10/17]
9. Altini M, Amft O. Estimating Running Performance Combining Non-invasive Physiological Measurements and Training Patterns in Free-Living. *Conf Proc IEEE Eng Med Biol Soc* 2018;2018:2845-48. doi: 10.1109/EMBC.2018.8512924 [published Online First: 2018/11/18]
10. Banister EW, Calvert TW. Planning for future performance: implications for long term training. *Can J Appl Sport Sci* 1980;5(3):170-6. [published Online First: 1980/09/01]
11. Morton RH, Fitz-Clarke JR, Banister EW. Modeling human performance in running. *J Appl Physiol (1985)* 1990;69(3):1171-7. doi: 10.1152/jappl.1990.69.3.1171 [published Online First: 1990/09/01]
12. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17(5):405-24. doi: 10.1038/gim.2015.30 [published Online First: 2015/03/06]