Endurance exercise and the risk of cardiovascular pathology in men: a comparison between lifelong and late-onset endurance training and a non-athletic lifestyle - rationale and design of the Master@Heart study, a prospective cohort trial

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ABSTRACT

Introduction Low and moderate endurance exercise is associated with better control of cardiovascular risk factors, a decreased risk of coronary artery disease and atrial fibrillation (AF). There is, however, a growing proportion of individuals regularly performing strenuous and prolonged endurance exercise in which the health benefits have been challenged. Higher doses of endurance exercise have been associated with a greater coronary atherosclerotic plaque burden, risk of AF and myocardial fibrosis (MF).

Methods and analysis Master@Heart is a multicentre prospective cohort study aiming to assess the incidence of coronary atherosclerosis, AF and MF in lifelong endurance athletes compared to late-onset endurance athletes (initiation of regular endurance exercise after the age of 30 years) and healthy non-athletes. The primary endpoint is the incidence of mixed coronary plaques. Secondary endpoints include coronary calcium scores, coronary stenosis >50%, the prevalence of calcified and soft plaques and AF and MF presence. Tertiary endpoints include ventricular arrhythmias, left and right ventricular function at rest and during exercise, arterial stiffness and carotid artery intima media thickness. Two hundred male lifelong athletes, 200 late-onset athletes and 200 healthy non-athletes aged 45–70 will undergo comprehensive cardiovascular phenotyping using CT, coronary angiography, echocardiography, cardiac MRI, 12-lead ECG, exercise ECG and 24-hour Holter monitoring at baseline. Follow-up will include online tracking of sports activities, telephone calls to assess clinical events and a 7-day ECG recording after 1 year.

Ethics and dissemination Local ethics committees approved the Master@Heart study. The trial was launched on 18 October 2018, recruitment is complete and inclusions are ongoing.

Trial registration number NCT03711539.

INTRODUCTION

Cardiovascular disease remains the most common cause of death worldwide, with an estimate of 17.8 million deaths in 2017, accounting for 31.8% of all-cause mortality. There is comprehensive evidence supporting the health benefits of regular physical activity. From a cardiovascular perspective, regular exercise has been associated with a lower prevalence and better control of cardiovascular risk factors (CVRF) such as arterial hypertension, dyslipidaemia, obesity and diabetes. As such, it is not surprising that coronary artery calcium (CAC) scores on CT coronary angiography (CTCA), the risk of developing coronary heart disease as well as all-cause and cardiovascular mortality are lower in individuals engaged in domestic work, leisure-time physical activity, walking and cycling. Furthermore, in the presence of atherosclerosis, higher exercise tolerance was associated with lower cardiovascular event rates and all-cause mortality. Radford et al demonstrated that higher levels of cardiorespiratory fitness attenuate the risk of cardiovascular disease and that the amount of attenuation increases as the coronary atherosclerotic burden increases. Regular physical activity is therefore proposed as one
of the most effective preventive lifestyle interventions with 150 min of moderate-intensity aerobic training per week reducing adverse events from coronary artery disease (CAD) by 50%.

In the past decades, an increasing number of middle-aged and older individuals have been exceeding these recommendations, engaging in intense exercise and participating in mass endurance events. Currently, there is controversy about the prevalence of coronary artery disease in the higher end of the spectrum of physical activity. In 2008, Möhlenkamp et al reported higher CAC scores in marathon runners compared with risk-factor matched controls. The hypothesis of causality between prolonged high-intensity endurance exercise and coronary atherosclerosis was, however, limited by the high prevalence (52%) of former smokers in marathon runners and the unknown history of other risk factors. Nevertheless, recent studies have shown similar findings. In a group of middle-aged men engaged in recreational or competitive endurance exercise, Aengevaeren et al reported higher CAC scores and a higher prevalence of coronary plaques in participants with the highest lifelong exercise volume (>2000 metabolic equivalent (MET)-min/week) and with the highest dose of very vigorous exercise (≥9 MET). However, many participants were current (4.9%) and former (38%) smokers and had a family history of coronary heart disease (31.3%). The latter risk factors could have influenced lifestyle changes, leading to a compensatory higher exercise volume in those carrying a higher cardiovascular risk history. In a study by Merghani et al, master athletes, compared with non-athletic controls with an equally low Framingham risk score, were more likely to have coronary plaques and elevated CAC scores. These two last studies further emphasised a significant difference in plaque composition, with athletes having more calcified, less mixed and similar proportion of non-calcified plaques. These findings are relevant as calcified plaques are proven more stable. In contrast, non-calcified and particularly mixed plaques are more vulnerable and prone to rupture, hence carry a higher risk of major adverse cardiovascular events. This has recently been confirmed by DeFina et al, who showed that higher levels of physical activity were associated with more prevalent CAC but without a significant increase in all-cause and cardiovascular disease mortality rates during 10.4 years follow-up.

The differing degree of coronary artery disease, including plaque composition and outcome, in athletes suggests non-conventional pathophysiology. Cardiovascular risk factors, such as age, a current and past history of smoking and a history of dyslipidaemia, remain relevant. However, in cases of low cardiovascular risk profile, exercise-related hypertension, coronary spasms, disruption of laminar flow, inflammation and elevated parathyroid hormone (PTH) levels have been proposed as potential instigators of atherosclerosis. Perhaps a combination of all of the above is needed for coronary atherosclerosis to develop in physically active individuals. Using CTGA, Lin et al evaluated coronary morphology before and after an extreme 140-day race in eight runners. Four runners with cardiovascular risk factors (smoking, hypertension) had coronary atherosclerosis at baseline, which progressed after the race. The remaining four runners were free from coronary atherosclerosis at baseline and did not develop any new coronary plaques post-race. Cardiovascular risk factors and coronary plaques might, therefore, be prerequisites for exercise to induce further atherosclerosis.

Similar to CAD, the relationship between exercise and atrial fibrillation (AF) is not straightforward. Concerning exercise, both the intensity and volume of exercise must be taken into account.

On the one hand, low to moderate-intensity exercise has been associated with a decreased risk of AF. In 2008 Mozaffarian et al reported a lower incidence of AF in individuals aged 65 years or older performing low to moderate physical activity such as walking. In the Tromsø study, investigators prospectively examined the association between physical activity and hospital-diagnosed AF in over 20 000 adults and demonstrated a 19% lower risk of AF in moderately active individuals.

On the other hand, higher exercise volume increases the risk of AF. Several studies have reported a higher incidence of AF when endurance exercise was performed more frequently (ie, >4/week) and longer (ie, >5 hours/week) or when a lifetime history of >2000 hours was met. A 2009 meta-analysis calculated that endurance athletes were 5.3 times more likely to develop AF than controls. The importance of substantial lifetime exposure to high-intensity endurance exercise has also been illustrated in a 2018 paper by Opondo et al reporting that 10 months of dedicated high-intensity exercise training induces changes in left atrial structure and mechanical function but without electrical consequences in middle-aged adults.

Another potential exercise-related cardiac detriment is myocardial fibrosis (MF). A body of research has reported a heterogeneous profile of myocardial scarring in endurance athletes concerning prevalence, location, extent, aetiology and clinical significance.

In 2008, Möhlenkamp et al documented a higher than expected prevalence of MF, as defined by late gadolinium enhancement (LGE) on cardiac magnetic resonance (CMR) imaging, in ostensibly healthy marathon runners compared with age-matched control subjects (12% vs 4%). In subsequent studies, the prevalence of MF has ranged between 0% to 50% of athletes. Differing patterns and locations of myocardial scar have been described in athletes. A first distinction exists between a CAD pattern, defined as subendocardial or transmural scarring in a coronary perfusion area, and a non-CAD pattern of MF. As examples of a non-CAD pattern, subepicardial and midmyocardial lateral wall fibrosis of the left ventricle (LV) as well as MF at the interventricular septum and right ventricle (RV) insertion points have been reported. Based on these patterns, several
aetiologies of MF have been proposed. A CAD pattern of fibrosis has been associated with an increasing CAC burden and significant coronary luminal stenosis. However, in some cases of CAD pattern of MF, no coronary abnormalities were found, and non-atherosclerotic mechanisms were suggested (i.e., coronary spasm, increased thrombogenicity and coronary emboli).

Subepicardial/midmyocardial lateral wall fibrosis might be caused by prior myocarditis and hinge point fibrosis by RV overload during exercise. The latter is supported by the association with cumulative exercise exposure, such as years of training and the lifetime number of races and race distances.

Most research was conducted in asymptomatic athletes meaning that MF in athletes was more often than not an incidental finding. Further research demonstrated that the clinical relevance of myocardial scarring varies and depends in part on its location. A CAD pattern and non-CAD subepicardial/midmyocardial MF have been associated with potentially life-threatening arrhythmias such as non-sustained ventricular tachycardia, but also ventricular tachycardia leading to sudden death or an appropriate shock by an implanted defibrillator. Postmortem evaluation in 357 cases of sudden death in athletes revealed a strong association between LV fibrosis and sudden cardiac death. Furthermore, large areas of subepicardial fibrosis of the LV have been linked with progressive LV dysfunction. By contrast, RV insertion point fibrosis is considered a benign finding, although it has been associated with a lower right ventricular ejection fraction (RVEF) at rest.

The mentioned studies have focused on the detection of focal MF by use of LGE. In recent years native T1 mapping has emerged as a technique to measure extracellular volume, enabling diffuse fibrosis detection. Some studies comparing athletes to non-athletes have measured similar T1 and extracellular volume (ECV) values. Others have reported lower T1 and higher ECV values in athletes with values remaining within normal ranges. In clinical practice, the use of T1-mapping is particularly useful to detect diffuse MF in cardiomyopathies such as hypertrophic cardiomyopathy and dilated cardiomyopathy.

In conclusion, the health benefits of low and moderate intensity endurance exercise have been challenged at higher exercise loads. Carefully designed prospective studies using a multimodality approach in larger cohorts are needed to clarify the effect of prolonged and strenuous endurance exercise on the cardiovascular system. The Master@Heart study is a multicentre prospective cohort trial designed to assess the relationship between starting age of extensive endurance exercise, and as such lifetime dose, and the prevalence of coronary atherosclerosis, AF and MF in master athletes.

METHODS AND ANALYSIS

Study design

The Master@Heart (Master Athlete’s Heart) study is a multicentre (University Hospitals Leuven, University Hospital Antwerp and Jessa Hospital Hasselt) prospective cohort study, funded by the Fund for Scientific Research, Flanders (T003717N). The study schema is presented in figure 1.

Objectives and hypothesis

The principal aim is to assess the prevalence of mixed, calcified and soft plaques in lifelong endurance athletes compared with endurance athletes who started training later in life and healthy non-athletic controls. The primary hypothesis is that lifelong endurance exercise, more than late-onset training, is associated with a lower prevalence of mixed plaques than non-athletic controls. As secondary aims, the Master@Heart study will assess the association between lifelong endurance training, MF and AF. The hypothesis is that prolonged exposure to endurance exercise is associated with a higher risk of AF and MF. The hypotheses are illustrated in figure 2.

Study population and eligibility criteria

An online screening questionnaire (www.masteratheart.be; online supplemental appendix) will be used to obtain information on gender, age, weight, length, current and previous smoking behaviour, load and timing of current and prior sports participation, CVRF, medication intake, previous cardiovascular conditions and family history in subjects willing to participate in the Master@Heart study (see online supplemental appendix). The questionnaire was built using REDCap electronic data capture tools hosted at the KU Leuven. All subjects from this screening cohort will be evaluated for eligibility according to the criteria listed in box 1.

Only male individuals will be eligible for inclusion in the Master@Heart trial because of the higher lifetime risk of coronary heart disease and AF in men relative to women. Moreover, as the risk of developing coronary heart disease before the age of 40 is low, we opted to include men between 45 and 70 years old. To identify an athlete through our online questionnaire, we will use a definition based on the hours per week of endurance training. For runners, we have put forward a cut-off of 26 hours per week as a 2016 paper by Dawes and colleagues showed significantly more cardiac remodelling when performing >5 hours of exercise per week instead of <5 hours per week. Given the higher intensity of running than cycling, the inclusion criterion for cyclists is ≥8 hours of cycling per week. Since cardiac volumes were similar between sedentary individuals and subjects performing <3 hours per week of exercise, we have chosen a cut-off of <3 hours per week of physical activity to define controls.
Exclusion criteria
To provide a clear view of the impact of intense endurance exercise on the cardiovascular system, we have decided to minimise the potential impact of CVRF by excluding subjects with known CVRF from the study (box 1).
been associated with a higher prevalence of plaque calcification and greater progression of CAC without higher event rates, which has been interpreted as the ability of statins to modulate coronary plaques. This has been confirmed by serial intravascular ultrasound analysis showing that statin therapy was associated with plaque atheroma regression as well as the progression of plaque calcification. Hence, individuals using statins will be excluded from our study. Elevated blood pressure has been related to an increase in cardiovascular events and mortality. Individuals using antihypertensive drugs will, therefore, be excluded from the Master@Heart study. Finally, with regard to overweight and obesity, as a body mass index (BMI) between 24.26 and 27.21 did not confer a higher OR for CAC >10 Agatston units, we opted for a BMI cut-off of >27.2 kg/m² as an exclusion criterion.

From all eligible subjects based on the questionnaire, 600 will be sampled for inclusion. Sampling will be done randomly but stratified by current age (45–53 years, 54–62 years and 63–70 years) as well as age at which endurance training was started (Group 1—lifelong: subdivided in <20 years and 20–30 years, Group 2—late-onset: subdivided in 31–40 years and >40 years, Group 3—healthy non-athletes: NA). This will give equal proportions with regard to current age in all three groups and equal proportions with regard to starting age of endurance exercise in lifelong and late-onset athletes. As the prevalence of high-level endurance athletes is lower in older individuals, we opted for a participant distribution of three out of seven aged 45–53 years, three out of seven aged 54–62 years and one out of seven aged 63–70 years. The sampling and stratification strategy is illustrated in figure 3.

**Study procedures**

Baseline evaluation will include an overview of medical history, review of medication and supplements, physical examination, blood sampling for biochemistry and genotyping, resting 12-lead ECG, two-dimensional and dimensional and multidimensional ultrasound of the heart, and measurement of exercise capacity. Figure 3 shows the sampling stratification of the Master@Heart study by current age (45–53 years, 54–62 years and 63–70 years) and age at which endurance training was started (lifelong: <20 years and 20–30 years; late-onset: 31–40 years and >40 years; non-athletic controls: NA). For current age a proportion of 3/7 : 3/7 : 1/7 of individuals aged 45–53 years, 54–62 years and 63–70 years, respectively, was applied.
three-dimensional resting echocardiogram (TTE), carotid artery ultrasound, pulse wave velocity for arterial stiffness and non-invasive central blood pressure measurements using a Sphygmocor device, cardiopulmonary exercise testing (CPET) including 12-lead exercise ECG and maximal oxygen consumption measurement, dual-energy X-ray absorptiometry, CTCA scan and a 24-hour Holter monitoring in all 600 subjects. CMR imaging, including gadolinium contrast administration, will be performed in 210 randomly selected subjects, 70 from each group. Randomisation for CMR occurs at the initial sampling and stratification with a similar age distribution. This subgroup of study participants will also undergo cardiac imaging during exercise, using two-dimensional TTE (Antwerp and Hasselt) or CMR (Leuven). The sequence of investigations will differ between sites based on logistics.

To gain a broad cross-sectional view on the association between endurance exercise and cardiovascular pathology, subjects will be followed-up for a minimum of 1 year. Follow-up will consist of online tracking of sports activity using TrainingPeaks (Peakware, Boulder, USA). At 6 months, a telephone call will assess clinical events such as alteration in medication, the onset of AF, coronary interventions or major adverse cardiovascular events. Finally, at 1-year follow-up, a 7-day ECG-monitoring will identify AF and other arrhythmias.

A clinical report covering all relevant findings will be available to the patient and his general practitioner. For research purposes, all measurements and recorded events will be pseudonymised and stored in the REDCap database.

**Physical examination and blood samples**

The information derived from the physical examination and blood sampling, such as systolic and diastolic blood pressure, total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, will be used to assess the individual cardiovascular risk score.

**12-lead ECG**

A resting 12-lead ECG will be recorded and interpreted following the Seattle Criteria by a cardiologist with experience in sports medicine.62

**Sphygmocor**

Carotid–femoral pulse wave velocity is a well-established marker of arterial stiffness and is linked to total cardiovascular risk.63 Using a Sphygmocor XCEL device (Sphygmocor device, AtCor Medical, Sydney, Australia), the central blood pressure, the central arterial pressure waveform, central aortic pressures and pulse wave velocities will be measured, allowing assessment of arterial stiffness. Pulse wave velocity is determined by simultaneous measurement of the carotid and femoral pulse by tonometry and volumetric displacement. The transit time between the feet of the two waves is calculated. Distances are measured from the suprasternal notch to the top of the thigh cuff, the site of carotid tonometry and the site where the femoral artery could be cannulated by tonometry.64

**Dual-X-ray absorptiometry**

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

**Cardiopulmonary exercise testing**

Peak oxygen consumption (peak VO₂) will be determined using a continuous bicycle stress test. After a 5 min warm-up, resistance will gradually increase by 30 W every minute from an initial load of 60 W in cyclists and 30 W in runners and controls until exhaustion. ECG monitoring during the test will ensure analysis of heart rate, arrhythmias and repolarisation abnormalities. During the exercise test’s final stages, the respiratory gas exchange will be analysed using a breath-by-breath open-circuit spirometry system. Peak VO₂ will be determined as the highest 30 s average oxygen consumption. The first and second ventilatory threshold will be determined from respiratory gas analysis parameters.

**Two-dimensional and three-dimensional transthoracic echocardiography**

Two-dimensional and three-dimensional TTE will be performed using a Vivid E9 or E95 ultrasound system (GE Healthcare, Horten, Norway) with an active matrix single-crystal phased array transducer (GE M5Sc-D probe, GE Healthcare, Horten, Norway) and 1.5–4 MHz matrix-array transducer (GE 4Vc-D Matrix 4D cardiac probe, GE Healthcare, Horten, Norway). Cardiac morphology will be assessed, including end-diastolic volume, end-systolic volume, rendering ejection fraction (EF) for both ventricles, as well as right and left atrial volumes. The diastolic function will be assessed using established Doppler and tissue-Doppler parameters such as the E wave velocity, the A wave velocity, the A/E ratio, septal, lateral and averaged E’, E/E’, tricuspid regurgitation flow velocity and the S-D-A waves at the pulmonary veins. An 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 systolic function measures. 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 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.65 66

**Carotid artery ultrasound**

The presence of atherosclerotic plaques at the common carotid artery and internal carotid artery will be evaluated...
using a ProSound Aloka Alpha 6 (Aloka Holding Europe AG, Zug, Switzerland), a Philips Epic®5 (Philips Medical Systems, Bothell, Washington, USA) or a Vivid E9 (GE Healthcare, Horten, Norway) ultrasound system with a 12L-3 (Philips Medical Systems, Bothell, Washington, USA) or Vivid E9 (GE Healthcare, Horten, Norway) or a 9L (GE Healthcare, Horten, Norway) transducer. Pulse wave Doppler measurement will be used to assess the degree of stenosis when plaques are present. To further assess peripheral atherosclerotic burden, the intima-media thickness will be measured as the distance between the hyperechogenic blood-intima line and the hypoechoic media-adventitia line at the distal part (1 cm proximal to the bulb) of the common carotid artery and the proximal part (1 cm distal to the bulb) of the internal carotid artery. All measurements will be made following international guidelines.67 68

CTCA

CTCA will be acquired using a 128-slice dual-source CT scanner (Siemens Somatom Force—Siemens Healthineers, Forchheim, Germany) or a 256-slice CT scanner (GE Revolution—GE Healthcare, Milwaukee, Wisconsin) or a 320-slice CT scanner (Aquilion ONE ViSION—Canon Medical Systems, Otawara, Japan). To achieve a target heart rate of <65 beats per minute, the beta-blocker esmolol will be used intravenously when necessary. The choice for esmolol instead of the more conventionally used beta-blockers (ie, metoprolol) was based on esmolol’s short half-life, which will prevent interference with other tests (ECG, CPET, TTE, CMR). All subjects will receive 0.4 mg sublingual nitroglycerine 2 min before scanning. First, a non-enhanced ECG-synchronised scan will be taken for the quantification of coronary calcium rendering the CAC score. Second, an ECG-triggered CTCA will be acquired after the intravenous injection of iodinated contrast medium in an antecubital vein followed by a saline chaser. All coronary atherosclerotic lesions will be analysed and interpreted following the 2016 Society of Cardiovascular Computed Tomography (SCCT) guidelines on syno.via (Siemens Healthineers, Forchheim, Germany) or GE Advanced Workstation (GE Healthcare, Milwaukee, Wisconsin) software with regard to their stenosis grade by the Coronary Artery Disease Reporting and Data System (CAD-RADS), composition (calcified, mixed, soft) as well as for their vulnerability (positive remodelling, low-attenuation plaque, spotty calcification and the napkin-ring sign). A density of >130 HU defines calcified areas. Calcified plaques are entirely composed of calcified areas. Mixed plaques are composed of both calcified and non-calcified areas. Soft plaques are entirely composed of non-calcified areas. The use of the syno.via Frontier Coronary Plaque Analysis software (Siemens Healthineers, Forchheim, Germany) will further assess luminal and plaque volumes.69–71

Cardiac MRI

In 210 randomly selected participants (70 per group, matched for age), CMR will be performed using a 1.5T MRI scanner (Magnetom Aera 1.5T—Siemens Healthineers, Erlangen, Germany; Ingenia, Achieva or Ambition 1.5T—Philips Medical Systems, Best, The Netherlands), a dedicated cardiac coil and electrocardiographic gating. Steady-state free precision short-axis cine imaging (8 mm slice thickness without gaps) will be obtained to analyse cardiac mass, function and volumes. Also, native and post-contrast T1 mapping will be performed using the modified look-locker inversion recovery sequence to calculate ECV. MF will also be evaluated using delayed enhancement of breath-hold phase-sensitive inversion recovery sequences 10 min after administering gadolinium-diethylenetriamine penta-acetic acid. Analysis of all CMR data will be performed in a central core laboratory. Assessment of cardiac volumes and mass will be performed using CVI42 (Circle Cardiovascular Imaging, Calgary, Canada). IntelliSpace Portal (Philips Medical Systems, Eindhoven, The Netherlands) will be used for T1 and ECV mapping, whereas suite-HEART (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.72 T1-mapping will also provide an estimate of myocardial cellular mass as a feature of athletic remodelling.73 In University Hospitals, native and post-contrast CMR imaging will also be performed during exercise. Our research group has previously demonstrated the feasibility, reliability and clinical utility of CMR to quantify cardiac volumes and function during exercise.74–76 The assessment of myocardial function during exercise will allow us to investigate whether the duration and intensity of long-term endurance exercise affect LV and RV functional reserve.

Exercise stress echocardiography

In the University Hospital Antwerp and Jessa Hospital Hasselt, exercise stress echocardiography (Vivid E95 ultrasound system—GE Healthcare, Horten, Norway) will be performed instead of exercise CMR in the subjects having undergone CMR at rest. Measurements will be performed at rest and during several exercise stages depending on heart rate and respiratory gas analysis parameters. The following measurements and derived calculations will be collected: left ventricular ejection fraction (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.77

Holter analysis

For the 24-hour Holter ECG monitoring, a Spider-view Holter device (ELA Medical, Paris, France) will be attached to BlueSensor VL ECG electrodes (Ambu, Penang, Malaysia). The ECG recordings will be analysed offline using SyneScope software (ELA Medical, Paris, France) to determine heart rate boundaries and to evaluate the occurrence of arrhythmias. Bradycardia is defined as a heart rate slower than 50 beats per min. A cardiac pause is defined as an interruption in the ventricular rate >2s and non-sustained ventricular tachycardia as three or more consecutive ventricular beats (origin below atrioventricular node) with an RR interval <600 ms (ie, >100 beats per min) and lasting <30 s.

Seven-day ECG monitoring

For the 7-day ECG monitoring, a RootiRx (Rooti Labs, Taipei, Taiwan) will be used. RootiRx is an ECG patch monitoring device consisting of an integrated sensor system, a microelectronic board with memory storage and an internal rechargeable battery. RootiRx allows for continuous ECG monitoring for up to 7 days in 250Hz frequencies with 24-bit high resolution. Recorded data is analysed by Rooti Labs developed algorithms and creates a report which is reviewed and edited by physicians and finally sent back to the referring physician. The final report includes the number of recording days, the amount of recorded beats, the average heart rate (overall, day and night), the maximum and minimum heart rate, the amount of cardiac pauses defined as an interruption in the ventricular rate >2s. AF burden is reported as an amount of AF events, time in AF and percentage time in AF. Reported atrial events include atrial ectopic beats and supraventricular tachycardia, and ventricular events include ventricular ectopic beats, doublets, triplets, bigeminy, trigeminy and ventricular tachycardia. The performance of RootiRx has been validated against standard 24-hour Holter monitoring in healthy individuals and patients with arrhythmias.78

Endpoints

The Master@Heart study’s primary endpoint is the difference in the prevalence of mixed plaques in lifelong endurance athletes, late-onset endurance athletes and non-athletic controls. The main secondary endpoints are (1) prevalence of AF on 12-lead ECG, a 24-hour Holter monitoring or a 7-day ECG-monitoring, (2) the presence and quantification of MF as assessed by LGE imaging (% of LV mass) and T1-mapping (% of ECV) and (3) total CAC scores and the presence of >50% stenosis in proximal coronary segments. Tertiary endpoints include: (1) prevalence of ventricular ectopic beats, non-sustained
and sustained ventricular tachycardia, a 24-hour Holter monitoring or a 7-day ECG monitoring; (2) LV and RV systolic function at rest and during exercise; (3) LV diastolic function by two-dimensional echocardiography including speckle tracking imaging; (4) bi-atrial function by two-dimensional echocardiography including speckle tracking imaging; (5) filling pressures and pulmonary artery pressures during exercise assessed by exercise echocardiography; (6) arterial stiffness by Sphygmocor; and (7) carotid intima media thickness as assessed by carotid artery ultrasound.

**STATISTICAL CONSIDERATIONS**

**Sample size and power calculations**

The Master@Heart study is powered to assess the association between the number of years (exercise age) middle-aged men have performed endurance exercise and the probability of mixed coronary plaques. The sample size calculations are based on the likelihood ratio $\chi^2$ test. Exercise age was assumed to be uniformly distributed between 0 and 40 years. A univariate logistic regression assessed the association between exercise age and the probability of mixed plaques. It was assumed that exercise can be assessed in the logistic regression as a linear, untransformed variable, that is, $\log(p/(1-p))=\alpha+\beta\times$exercise age. Statistical testing is two-sided and assessed at a significance level of 5%. PROC POWER in SAS V.9.4 (SAS/STAT V.14.1) was used to calculate the sample size for a univariate logistic regression with exercise age as the only covariate. In line with recent research, the anticipated incidence of mixed plaques is 35% in subjects with an exercise age of 0 years (P0) and 22% in subjects with an exercise age of 25 years (P25).18 19

**Table 1** Demographic and physical activity data on eligible lifelong, late-onset athletes and healthy non-athletes

<table>
<thead>
<tr>
<th></th>
<th>Non-athletes (n=218)</th>
<th>Lifelong (n=436)</th>
<th>Late-onset (n=282)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>54.2±6.5</td>
<td>53.5±5.9</td>
<td>54.96±6.3</td>
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<tr>
<td>Weight (kg)</td>
<td>76.0±8.3</td>
<td>74.6±7.4</td>
<td>75.3±8.1</td>
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<td>Length (cm)</td>
<td>178.3±6.5</td>
<td>179±6.1</td>
<td>179.3±6.2</td>
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<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>23.9±1.9</td>
<td>23.3±1.7*</td>
<td>23.4±1.9</td>
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<td>Endurance exercise (hours/week)</td>
<td>1.5±1.3</td>
<td>11.6±3.8*</td>
<td>11.3±4.2*</td>
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<tr>
<td>Total physical activity (hours/week)</td>
<td>1.6±1.3</td>
<td>11.8±3.9*</td>
<td>11.4±4.2*</td>
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<td>Start age endurance exercise (years)</td>
<td>NA</td>
<td>17.6±6.6</td>
<td>40.7±6.4†</td>
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</tbody>
</table>

Demographic and physical activity data, based upon the screening questionnaire. Values are mean±SD. *P<0.05. Unpaired analysis of variance comparison, significantly different from healthy non-athletes. †p<0.05. Unpaired comparison, significantly different from lifelong athletes.

**Figure 6** Distribution of endurance, cycling and running training hours. Panel (A) depicts the distribution of endurance exercise hours per participant who filled in the screening questionnaire including the average training hours for controls and athletes as per the inclusion criteria. Panels (B) and (C) show the distribution of running hours per week (B) and cycling hours per week (C) of participants who filled in the screening questionnaire. The black frames indicate the participants that fulfil the criteria for athletes as per the study inclusion criteria.
Under these assumptions, a sample size of 486 subjects would offer a statistical power of 80% with a 5% probability of a type I error. In the Master@Heart study, 600 subjects (200 per group) will be included, which will give a margin of up to 20% uninterpretable data.

Analysis of variance and non-parametric approaches will be used to analyse continuous outcomes. For dichotomous outcomes, $\chi^2$ tests will be used. The relationship between calcium score and current and historic exercise load will be assessed using Pearson or Spearman correlation as appropriate. Multiple linear, non-linear and logistic regression analysis will be used to determine the association between coronary atherosclerosis or AF and endurance exercise load and cardiovascular risk factors. Losses to follow-up are expected to be minimal as extensive phenotyping is performed at baseline. A subsequent follow-up will consist of telephone calls and a single 7-day heart rate monitoring.

TRIAL STATUS

The Master@Heart study was officially launched on 18 October 2018 with a press event organised in collaboration with Golazo (Paal-Beringen, Belgium). Golazo is an organiser of sporting events in Belgium and promotes all aspects of physical activity. On the same day, our website and online registration tool were launched (www.masteratheart.be). Potential candidates could register and received a personal code that provided access to an online questionnaire linked to the REDCap database (online supplemental appendix 1). The information from the questionnaire was pseudonymised. The launch resulted in a peak of registrations in the first month, after which a decline in registrations was observed until July 2019 (figure 4). Therefore, in the second half of 2019, a social media campaign was set up in combination with the distribution of flyers at several national sporting events, which led to an increase of registrations until January 2020.

As of May 26 2020, 3711 registrations were obtained, of which 3615 (97%; 3389 men) were completed correctly (figure 5). Out of these 3389 male participants, 1933 were excluded based on the exclusion criteria specified in box 1. As depicted in figure 6, 936 of the remaining 1456 participants based on the questionnaire (online supplemental appendix) fulfilled the inclusion criteria for physical activity. These eligible participants will be sampled after stratification by (1) age at which endurance training was started and (2) current age. Non-athletes had a slightly higher BMI ($23.9\pm1.9$ vs $23.3\pm1.7$ kg/m$^2$, $p<0.05$, table 1) than lifelong athletes. No anthropometrical differences were observed between lifelong and late-onset athletes. Per inclusion criteria, the amount of endurance exercise per week in non-athletes was lower than in late-onset and lifelong athletes ($1.5\pm1.3$ vs $11.3\pm4.2$ vs $11.6\pm3.8$ hours/week, respectively; $p<0.05$).

The first participant gave informed consent and took part in the study on 21 February 2019, and recruitment is expected to finish in 2021 with a delay of 6–12 months due to the COVID-19 pandemic. Trial results will be communicated through publications. Published articles will be available to the community on www.masteratheart.be.

CONCLUSION

A better understanding of the relationship between intense and prolonged endurance exercise and atherosclerosis, MF, arrhythmias and other cardiovascular diseases in middle-aged and older athletes is needed. Athletes have increased longevity but are not granted immunity from cardiovascular disease. Whether or not there is a threshold at which the risks of endurance training outweigh the benefits remains unanswered.

The rigorous and extensive phenotyping performed in The Master@Heart trial will provide insight into the exercise-dose response of prolonged and intense endurance training on the whole cardiovascular system. This will enable clinicians better to gauge the risk of cardiovascular disease in athletes and guide future clinical management.

Acknowledgements The authors would like to thank the many staff members at the three sites for helping conduct this study. We would particularly like to thank the clinical research assistants Sofie van Soest, Dorien Vermeulen and Daisy Thijs for their dedication and devoted efforts to include, test and follow-up participants.

Contributors All authors contributed to the design of the Master@Heart trial. RDB, GC and AB developed the sample size calculation and the statistical analysis plan. RDB, CD, GC and RW drafted the manuscript. All authors read, gave final approval and agreed to be accountable for all aspects of the work, ensuring integrity.

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Competing interests RW is supported as a postdoctoral clinical researcher by the Fund for Scientific Research Flanders (FWO Vlaanderen).

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval The KU Leuven Biomedical Ethics Committee and the committees of UZ Antwerpen and Jessa Zielenhuis Hasselt approved the study (reference B322201837094).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement No data are available.

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REFERENCES
Appendix

1. **Online Master@Heart Questionnaire**

The Master@heart questionnaire was developed using Redcap in Dutch. The questionnaire is accessible via: [https://redcap.gbiomed.kuleuven.be/surveys/?s=FJX8DY74TA](https://redcap.gbiomed.kuleuven.be/surveys/?s=FJX8DY74TA)

The link is only available after completion of a registration on the website and using the registration code sent to the athlete. This way of workings allows strict pseudonymisation. Redcap makes use of several question options, for example an open field or dropdown menu with options available. Behind the question, in brackets, the used option is depicted and the options are displayed. Some questions are branched, which means they are only visible when another question is answered. If, for example, you never smoked, you will not see other questions involving past or present smoking behaviour.

The participants are asked to truthfully complete the questionnaire.

**Questionnaire**

1) Provide your registration code, make sure to use capitals when appropriate. (*Open field*, if the code corresponds to the database the next questions will appear)

2) I agree that the provided data can be used confidentially for scientific research. The data can only be used in the context of this study. The data will be coded so that they cannot directly be linked to your identity. (*Drop down*, Agree or not agree)

3) I agree to be contacted to participate in the research project, which includes several examinations for the heart. These include a coronary CT, a cardiac MR, if I get selected. This is voluntary and without any commitment. (*Drop down*, Agree or not agree)
Demography

4) Sex (Drop down, male or female)

5) Birthday (Date field)

6) Date of registration (Date field)

7) Highest education (Drop down, primary school; high school; university; I still go to school)

8) Do you work? (Yes or no)
   a. Do you work with fixed hours? (Drop down, Yes; No I work in shifts; No I work in shifts including night shifts; No I’m self-employed)
   b. How many hours do you work per week? (Drop down, 1;2;…,>42)

9) What is the name of your personal physician? (Open field)

10) What is your length in cm? (Open field)

11) What is your weight in kg? (Open field)

12) Do you smoke? (Drop down, I never smoked; I quit smoking; I still smoke)
   a. If you quitted smoking.
      i. How long ago did you stop smoking? (Drop down, less than 5 years; less than 10 years; more than 10 years; more than 20 years)
      ii. How long did you smoke? (Drop down, less than 1 year; less than 2 years; less than 3 years; less than 5 years; less than 10 years; more than 10 years)
   b. If you still smoke
      i. How long do you smoke? (Drop down, less than 1 year; less than 2 years; less than 3 years; less than 5 years; less than 10 years; more than 10 years)
13) Do you take medication for diabetes? *Yes or no*

14) How many alcoholic beverages do you drink per week? *Drop down, I drink no alcohol; 1; 2; 3; 4;…; >14)*

**Current sport activity**

15) Do you, at this moment, participate in weakly sport activities? *Yes or no*

a. If yes, which sports do you do weekly? *Multiple answers possible*

   i. Cycling
   ii. Running (>1500m)
   iii. Running (<1500m)
   iv. Triathlon (If selected questions for swimming, running and cycling will be displayed)
   v. Swimming
   vi. Football
   vii. Basketball
   viii. Handball
   ix. Golf
   x. Chess
   xi. Dancing
   xii. Gymnastics
   xiii. Omni sport
   xiv. Rowing
   xv. Darts
   xvi. Weight lifting, powerlifting
   xvii. Badminton
xviii. Tennis

xix. Duathlon (If selected questions for running and cycling will be displayed)

xx. Fighting sports

xxi. Other (specify this sport in an open field)

xxii. Volleyball

b. For every sport selected above the following questions will appear:
   
i. At which age did you start (selected sport)? (Drop down, 1; 2;...; 69)
   
ii. Did you ever stop (selected sport) longer than 3 years? (Yes or no)
   
iii. Do you participate in (selected sport) for over half a year? (Yes or no)
   
iv. At which level do you participate in (selected sport)? (Drop down, recreationally; recreational competition, competition regionally; competition nationally; competition international)
   
v. How many hours do you train for the (selected sport)? (Drop down, 1; 2;...; >30)
   
16) Do you perform strength training in a fitness centre? (Yes or no)

   a. If yes, how many strength-training do you perform (Drop down)
      
i. 1 time per week
   
ii. 2 times per week
   
iii. 3 times per week
   
iv. 4 times per week
   
v. 5 times per week
   
vi. 6 times per week
   
vii. Daily

17) If you work, how do you go to work? (Drop down)
a. Public transport
b. Car
c. Bike
d. Running
e. Walking
f. Cycling or running with car or public transport
   i. If you cycle or run, how long do you run or cycle to work each week?
      (Drop down)
         1. <1 hour
         2. >1 hour
         3. >2 hours
         4. >3 hours
         5. >4 hours
         6. >5 hours
         7. >6 hours
         8. >7 hours
         9. >8 hours
        10. >9 hours
        11. >10 hours

18) If you still go to school, how do you go to school? (Drop down)

   a. Public transport
   b. Car
   c. Bike
   d. Running
   e. Walking
f. Cycling or running with car or public transport
   
   i. How long do you run or cycle to school each week? *(Drop down)*
      
      1. <1hour
      2. >1hour
      3. >2hours
      4. >3hours
      5. >4hours
      6. >5hours
      7. >6hours
      8. >7hours
      9. >8hours
     10. >9hours
     11. >10hours

Past sport activity

If you in the past followed an education, which included sport lessons as an important part of your education, please do not count this as sport in the past, unless this was sport for training purposes in a sport school.

19) Which sports did you perform in the past? *(Multiple answers possible)*

   a. Cycling
   
   b. Running (>1500m)
   
   c. Running (<1500m)
d. Triathlon (If selected questions for swimming, running and cycling will be displayed)

e. Swimming

f. Football

g. Basketball

h. Handball

i. Golf

j. Chess

k. Dancing

l. Gymnastics

m. Omni sport

n. Rowing

o. Darts

p. Fitness

q. Weight lifting, powerlifting

r. Badminton

s. Tennis

t. Duathlon (If selected questions for running and cycling will be displayed)
u. Fighting sports

v. Other (specify this sport in an open field)
w. Volleyball

x. I never participated in sports

i. For every sport selected above the following questions will appear:

1. At which age did you start (selected sport)? (Drop down, 1; 2; 3;…; 70)
2. At which age did you stop (selected sport)? *(Drop down, 1; 2; 3; …; 70)*

3. At which level did you participate at this sport? *(Drop down, recreationally; recreational competition, competition regionally; competition nationally; competition international)*

4. How many hours did you train per week for (selected sport)? *(Drop down, 1; 2; 3; …; 30)*

   ii. If fitness, how many times per week did you go to a fitness centre for strength training. *(Drop down, 1; 2; 3; 4; 5; 6; 7)*

**Health questions**

20) Have you ever been examined or treated for chest pain or breathlessness at rest? *(Yes or no)*

21) Have you ever been examined or treated for chest pain or breathlessness during exercises? *(Yes or no)*

22) Have you ever been examined or treated for palpitations or cardiac arrhythmias? *(Yes or no)*

   a. If yes, did this involve atrial fibrillation or atrial flutter? *(Yes or no)*

   b. Did you receive a pacemaker or defibrillator? *(Yes or no)*

23) Have you ever been examined or treated for dizziness during or after exercise? *(Yes or no)*

24) Have you ever fainted during or after exercise? *(Yes or no)*

25) Has a doctor ever mentioned you have a heart murmur? *(Yes or no)*

26) Has a doctor ever mentioned you have an elevated blood pressure? *(Yes or no)*

27) Do you take medication for an elevated blood pressure? *(Yes or No)*

28) Has a doctor ever mentioned you have high cholesterol levels? *(Yes or No)*
29) Do you take medication for high cholesterol levels? *(Yes or No)*

30) Are you known to have problems of the coronary arteries? *(Yes or No)*

31) Do you have other complaints of the heart or blood vessels? *(Yes or no)*
   
   a. If yes, which? *(Open field)*

32) Do you take medication at this moment? *(Yes or no)*
   
   a. If yes, which? *(Only mention the name; open field)*

33) Have you even been diagnosed with asthma? *(Yes or no)*

34) Have you ever suffered from coughing, shortness of breath or breathing disorders during or after exercise? *(Yes or no)*

35) Do you use or have you used inhalation medication? *(Yes or no)*

36) Do you have any allergies (pollen, medication, food, insects)? *(Yes or no)*
   
   a. If yes, which? *(Open field)*

37) Other lung problems? *(Yes or no)*
   
   a. If yes, which? *(Open field)*

**Family disorders**

38) Has a stroke or myocardial infarction before the age of 65y occurred in a family member? *(Yes or no)*

39) Has sudden death before the age of 50y occurred in a family member? *(Yes or no)*

**Participation in the study**

40) In which testing centre would you like to undergo testing? You can provide multiple answers. *(Drop down, UZ Leuven; UZ Antwerpen; Jessa Hospital Hasselt)*

41) Do you suffer from claustrophobia? *(Yes or no)*
2. **Master@Heart Informed Consent**

This informed consent was translated from Dutch to English.

**PATIENT INFORMATION AND CONSENT FORM MASTER AT HLETE'S HEART STUDY (MASTER @ HEART)**

**Patient information and consent form**: Non-athletes and endurance athlete performing at recreational / national / international level, age older than 45 years.

**Date of this version**: Version 3 (9-10-2018)

**Project Title**: Endurance exercise and the risk of cardiovascular pathology in men. A comparison between lifelong and late-onset endurance training and a non-athletic lifestyle

**Study group**: Non-athletes and endurance athletes

**Institution**: KU Leuven

**Principal Investigators**: Prof. Dr. Rik Willems, Dr. Guido Claessen, Prof. Dr. Hein Heidbuchel, Dr. Lieven Herbots

**Co-investigator(s)**: Mr. Christophe Dausin, Mrs. Jessica Ratajczak, Mrs. Daisy Thijs, Dr. Ruben De Bosscher, Dr. Mathias Claeyts, Prof. Dr. Jan Bogaert, Prof. Dr. Steven Dymarkowski, Prof. Peter Hespel, Dr. Kaatje Goetschalckx, Prof. Dr. Andre La Gerche, Prof. Dr. Barati Shivalkar, Dr. Caroline Van De Heyning, Prof. Dr. Paul Dendale, Prof. Bert Op t' Eijnde, Dr. Olivier Ghekiere

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This consent form is 11 pages long. Read all pages.

1. Your Consent

You are invited to participate in a research project.

This consent form contains detailed information about the research project. The intention is to explain to you as clearly as possible the purpose of the study and the investigations it will require. This way you can make a well-informed decision on whether you want to participate or not.

Please read this consent form carefully. Feel free to ask additional questions to the involved researchers. You may discuss your consent to participate in the project with a family member, friend or your GP.

If you decide to participate, you will be asked to sign at the bottom of this consent form. By signing this form you indicate that you have understood the information in this document and that you wish to participate in the research project.

You will receive a copy of this consent form.

2. Purpose and background

It is well known that endurance training has a positive effect on health and life expectancy. However, to date no studies have determined the optimal dose of endurance training. It is possible that exceeding of a certain exercise dose is accompanied by an increase in the risk of cardiac conditions such as arrhythmias.

The goal of this project is to compare the impact of lifelong exercise on the heart as compared with exercise initiated later in life and a healthy lifestyle without exercise. A total of 600 volunteers will be asked to participate in this research project. The total study population will be divided into 3 groups according to the history of endurance sports (lifelong endurance sports versus late onset endurance sports versus no endurance sports). Specifically, we want to see if lifetime exercise is associated with a reduction in the risk of coronary artery disease and whether this is at the expense of a higher risk of arrhythmias and scarring of the heart muscle. We want to investigate whether starting endurance training at a later age has the same benefits as starting at a younger age. We hope to lay a scientific basis for training programs that stimulate endurance sports in Flanders.

If we can find out which underlying causes in the athlete lead to side effects of endurance sports, we could implement specific preventive and therapeutic measures in predetermined athletes. To support this project, a collaboration was set up with various national sports authorities (Sport Flanders, Royal Belgian Cycling Federation, Flemish Triathlon and Duathlon League and Association for Sports and Examination Physicians) in the form of an advisory board. The aim of this collaboration is to translate the research results of the project into guidelines for endurance training and the implementation of sound expensive sport to stimulate, in our society down.

This research is carried out by Belgian experts from different hospitals (UZ Leuven, UZ Antwerp and Jessa Hospital Hasselt). In these different centers there is extensive experience in studying the athlete’s heart. This research project is the most comprehensive to date tackling the benefits and potential detriments of endurance exercise. However, we realize that participation also requires an important commitment from you.
Below, we will explain how this study utilized imaging of the heart through X-ray, (coronary CT), ultrasound (echocardiography) and magnetic resonance imaging (MRI). During a long planning phase, we weighed up the various elements of this research project with many experts. Although we realize that it takes a considerable effort on your part to participate, we believe it provides the best opportunity to answer the research questions.

3. Procedures

The investigations will require a full day of two half days of your time at baseline. Follow-up will require a short visit to the participating center. If you participate in this study, we will draw up the most appropriate plan with you. As a result, the order of the studies listed below may vary. An additional cardiac MRI will be performed for some of the participants. The selection of these participants is random.

History, clinical examination and electrocardiogram (30 minutes)

During this first visit you will be asked about your medical history and any health complaints. You will be asked to fill in a questionnaire regarding your sports activities. You will be weighed and clinically examined. Next, will be perform an electrocardiogram (an examination in which the heart rate is measured by means of a number of electrodes on the skin).

Blood samples for storage

First, an ordinary blood sampling will be done (+/- 30ml) where a small catheter is introduced on the inside of the arm. The latter will be used to administer contrast fluid during the coronary CT (and cardiac MRI, if this will be performed on you). For people who undergo a cycling test in the MRI, a second amount of blood (+/- 10 ml) will be taken before and immediately after the exercise via the same catheter. With blood sampling there is a small chance of local pain and a small bruise afterwards. The presence of the catheter is associated with minimal discomfort. At the end of the examinations (coronary CT and possibly cardiac MRI) the catheter is removed, which is also a quick and almost painless procedure. A patch is then applied to the insertion site, which can be removed the day after.

From the blood samples we will measure troponin (a substance that is released when the heart is damaged), and NT- proBNP (a substance that is released if the heart is overloaded). We also ask your permission to store small amounts of blood for further research regarding the link between coronary artery problems and certain substances associated with inflammation and the degree of heart muscle remodeling in relation to certain genetic characteristics. In particular, we want to investigate whether certain genetic variants are associated with the development of more extreme remodeling of the heart as a result of intensive endurance exercise and whether this is associated with beneficial or possibly adverse effects on performance and health in the long-term.

If you give your consent, we will draw a small amount (approximately 20 ml) of blood from which DNA and RNA will be isolated and which will be stored in a biobank for 30 years. The blood samples will be stored in encrypted form so that they cannot be traced back to you by third parties. The code that relates to the identity data of the DNA samples is managed by the Center for Human Genetics at UZ Leuven. They may be exchanged with other laboratories as part of a scientific collaboration, without commercial connotation, with the agreement of the ethics committee.

Research of the genetic material in the context of this biobank is intended to enable scientific research, but not to determine a genetic disorder or to determine the risk thereof. Normally, the results will therefore not be communicated to you. However, if the studies performed on your
biological material provide information that has a significant effect on your health condition, your treating physician will be advised and will discuss it with you. In addition, you will be offered genetic counseling and clinical follow-up for further follow-up. No decisions about eligibility for sports will be made solely on the basis of genetic testing. These findings will always be combined with the other test results.

All blood samples that are not used for the biobank, or for which no permission is given for storage in the biobank, will be destroyed after analysis. In principle, within 3 days. The stored genetic material will only be used in the above-mentioned context. Undefined research on collected samples will be defined in a new protocol and may only be started after approval of the Ethics Committee. You will not be contacted again for this.

If you withdraw your consent to participate in the study, you can have your sample(s) destroyed or requested back. Please contact the investigator for this. The results obtained from your sample(s) before you withdraw your consent to participate will remain the property of the client.

If you have any questions, please contact the coordinator of the biobank (UZ Leuven):
Prof. Dr. Nadine Ectors
UZ Leuven - Gasthuisberg Campus
gray, floor 0, Biobanking room
Herestraat 49, 3000 Leuven
Tel.: +32 16 345 485
e-mail: wbb@uzleuven.be

Cardiac MRI and blood test (60 minutes; with a limited number of participants)

As already mentioned, a cardiac MRI will only be performed on some of the participants. The selection of these persons is completely random.

The cardiac MRI will be performed on the Magnetic Resonance unit. MRI is a specialized examination that uses magnetic fields to obtain a very detailed image of your heart. This part of the examination does not require any radioscopy. Since large magnetic fields are used, it is essential that you do not have any implanted metal object (such as a pacemaker, defibrillator, prosthesis, artificial valve,…). Your researcher will verify by means of a questionnaire that none of these contraindications exist and that you can undergo the MRI examination without risk. MRI is a very safe study. For the scan you will be placed in a fairly narrow bore, which is initially a bit frightening for some patients but you will get used to this quickly.

A small dose of contrast (Gadolinium) will also be administered during this examination. This substance has special magnetic properties that allow it to clearly distinguish between the heart and the heart tissues, and to indicate even small areas of scar tissue. Side effects are also very rare. Sometimes the administration leads to mild headaches or nausea, which usually clears up quickly.

The MRI we use in this study is specially equipped with a supine bicycle. After the imaging, you will be asked to exercise on this bicycle at rest. This will feel strange because of the reclining position, but otherwise this is no different from normal exercise. First you will be asked to cycle at a comfortable pace for 2 minutes. You will then be asked to cycle for 2 minutes at a heavier resistance and finally 2 minutes at near maximum intensity. You will receive precise instructions for this during the examinations.

Coronary CT (30 minutes)
A coronary CT will be performed on all participants. In the CT examination room, you will lie on your back on the examination table as comfortably as possible. An IV will be placed in the vein of your arm to administer medication and contrast medium. Electrodes are placed on the chest to monitor the heart rhythm. In order to make a good CT scan, the heart rate must be relatively low, preferably below 65 beats per minute. If you still have a heart rate above 65 beats per minute in the initial phase of the CT examination, you will receive medication (beta blocker) through the IV into the vein of your arm. The coronary arteries must also be dilated. Therefore you will receive a single spray of nitrate underneath your tongue before the start of the examination. The medical team is located just outside the examination room during the recordings and can see and hear you perfectly. From time to time we will ask you to hold your breath.

These preparations for the examination are identical to the daily routine of the CT examination of the coronary arteries and are therefore not affected by the study.

In a CT examination, thin cross sections of the body part to be examined are made using X-rays, in this case the chest and heart. During the examination, you will slide through a ‘ring’ while lying down. The tube is large enough so that this is not a problem if you suffer from claustrophobia. A contrast medium is used to properly image the heart and the coronary arteries (CT angiography or CTA).

**Echocardiography at rest (40 minutes)**

In this exam, an ultrasound probe will be placed on your chest (after applying a special gel). This allows images of the heart to be taken from different directions. This examination is completely painless and safe. You may have already experienced this in the past. You will be asked to turn to the left side and hold your breath occasionally.

**DXA scan (10 minutes)**

A DXA scan will be performed to find out more about your body composition (the amount of bone, fat and muscle in the body). For this examination you will be asked to lay your back on an examination table. During the examination, which takes about 7 minutes, a very low dose of X-rays will pass through the body. You will not notice any of this yourself.

**Carotid artery ultrasound and measurement of arterial stiffness (30 minutes)**

In this study an ultrasonic probe images takes imaged from the carotid artery where the blood vessel wall thickness can be measured as a measure of atherosclerosis. This technique is similar to the ultrasound of the heart and is completely painless. We will also estimate the stiffness of the vascular system by means of pulse wave analysis. In this test, three electrodes are placed on the skin and the distance is measured from the sternum to the cervical and thigh artery. The pulse wave velocity is then measured at the level of the carotid artery and the thigh artery with a special probe.

**Maximum exercise test (30 minutes)**

You will be asked to perform a maximum exercise test in order to measure maximal oxygen consumption by your body, as a measure of your fitness. This is can be done running on treadmill or by cycling of a bike (which can even be your own bike). The choice of the exercise test will depend on the endurance sport you practice. Gradually, the resistance of speed will be increased until you are no longer able to continue. During the exercise test you will take in a mouthpiece that can analyze the exhaled air and thus measure the oxygen consumption. At the start of the test a small puncture wound in your earlobe will allow us to collect a drop of blood.
(2 to 5 μL) every 4 minutes during the test, on which we will measure lactate (~ lactic acid). This injection hardly hurts. This allows us to determine your exercise capacity. Your heart rhythm will also be continuously monitored during this test. At the end of the examination, you be able to cool down at your own pace. With the presence of a qualified cardiologist and all necessary equipment, the risk is minimal, and in any case many times smaller than with efforts (even less intense) in daily life.

**Holter monitoring**

After completing the studies, you will be asked to record the electrical activity of the heart for 24 hours using a (compact) portable device (Holter ECG). During that period, the equipment continuously records your heart rate. The records show the course of your heart rhythm over 24 hours and allows us to visualize any irregularities in the heart rhythm. After 24 hours, you can simply remove the holter and send it back.

**Follow-up and repetition of investigations**

After completion of the examinations, your medical condition will be monitored for at least 1 year. You will be contacted by telephone every six months regarding your participation in sports activities and whether or not clinical problems occur. In addition, we will also ask you to create an online account on TrainingPeaks, a webpage that allows you to upload training data obtained with your heart rate monitor. Your permission will be asked to allow us access to your training data. We emphasize that these training data are strictly confidential and not aimed at providing training advice. This close monitoring makes it possible to determine whether the degree of cardiac muscle remodeling in the context of endurance sports is associated with beneficial effects on health in the long term and whether some athletes may have an increased risk of developing arrhythmias.

To detect arrhythmias, you will be asked to wear a monitor that measures your heart rhythm for several days 1 year after the start of the study.

Research that has not yet been defined will be defined in a new protocol and may only be started after approval by the Ethics Committee.

**Overview of the studies**

<table>
<thead>
<tr>
<th>Procedure</th>
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<tbody>
<tr>
<td><strong>Day 1</strong></td>
</tr>
<tr>
<td>• Completing the questionnaire and clinical examination</td>
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<tr>
<td>• Resting ECG</td>
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<tr>
<td>• DXA scan</td>
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<tr>
<td>• Echocardiography at rest</td>
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<td>• Duplex neck vessels and arterial stiffness measurement</td>
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<tr>
<td>• Maximum cycling test with measurement of oxygen consumption and ECG registration</td>
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<tr>
<td>• Blood taking</td>
</tr>
<tr>
<td>• MRI of the heart at rest (only for a limited number of participants; optional: during exercise)</td>
</tr>
<tr>
<td>• Coronary CT</td>
</tr>
<tr>
<td>• Adhering to 24h holter monitor</td>
</tr>
<tr>
<td>END of examinations day 1</td>
</tr>
</tbody>
</table>
### Day 2
- Return the 24-hour holter monitor (or send it to us in consultation with the study director)

### Clinical follow-up (1y)
- Telephone contact every six months to evaluate problems
- Synchronization of training data in TrainingPeaks
- Multi-day ECG registration 1 year after inclusion

### 4. Potential Benefits
We can’t promise any direct personal benefit for you from this research. Still, some interesting findings may be made for you:

- If there are abnormalities in heart function or electrical function, it is likely that it can be picked up during these tests. The proposed studies in this study represent the most accurate evaluation of cardiac function currently available. If abnormalities are found during the investigation will assess with you and your GP the possible need for additional tests and follow-up.

- In addition, we hope that this research can allow a better understanding of the relationship between endurance sports and heart disease in some patients. Through better preventive and diagnostic tests, you can therefore help future athletes to diagnose and prevent health problems in a timely manner.

### 5. Possible risks
- Because of the effort: Vigorous effort carries a small risk of provoking an arrhythmia. Previous studies have shown that the risk of sudden death is about 1 to 2 cases per 100,000 athletes per year and at 1 per 1 to 4 million hours of exercise. On the other hand, the consequence of this is much lower in a controlled environment than if it were to occur in daily life. The risk of injury or death is extremely low under these circumstances. Should an arrhythmia occur, it can be treated immediately and will also lead to a change in policy. During all exercise tests is an experienced cardiologist will be present, who can immediately recognize and treat any problem.

- Coronary CT: In a CT of the heart and blood vessels, an iodinated contrast agent is administered through a vein, usually in the elbow crease. This serves to make the heart chambers, coronary arteries and / or abnormalities more visible. In most cases, the administration proceeds without problems. You may feel warm for a short time or feel slightly nauseous. In a small number of patients (less than 1%) an allergic reaction to the contrast medium occurs, which usually consists of sneezing or the appearance of red patches on the skin. In most cases, no further treatment is required for this. A serious allergic reaction is extremely rare (less than 1: 10,000 cases). The employees of the radiology department in the various research centers know how to act in such a special situation. If you have had an allergic reaction during a previous examination with iodinated contrast agent, we request that you inform your cardiologist and / or radiologist in advance. The examination is carried out with as little X-rays and contrast medium as possible to obtain good quality images. The CTA exam usually requires 60 ml of contrast medium and the X-rays range between 0.5 and 1.5 mSv. By way of comparison: the annual natural background radiation in Belgium is 4 mSv per year.
• **Magnetic Resonance Imaging (MRI):** There is a small risk of headache or nausea from the contrast medium. Allergic reactions have been described extremely rarely. Some people feel trapped in the MRI scanner. If this is really unbearable, you will of course be immediately taken out of the scanner.

• **DXA scan.** A DXA scan uses X-rays. However, the dose is extremely low and less than two days of exposure to natural background radiation.

• **Echocardiography:** Ultrasound examination is a very safe examination. There are no risks associated with this research.

• There is a very small chance that the studies performed will reveal an abnormality of the heart that would otherwise have gone unnoticed and not necessarily caused any symptoms or danger. Such a finding can raise concern. Should such a finding nevertheless be made, we will inform you in detail about its importance. On the other hand, such a finding could be seen as a benefit, as it could lead to targeted treatment and follow-up, which could prevent symptoms in the future.

• Any investigation can involve unforeseen and unknown risks. Nevertheless, we believe that this is very unlikely given the extensive daily experience of all these studies.

### 6. Privacy, Confidentiality, and Information Disclosure

The confidentiality of this study is guaranteed like any other medical information. Any information obtained in connection with this research project that can identify you will be treated with the utmost confidentiality in accordance with EU Regulation 2016/679 (General data protection) on the protection of natural persons with regard to the processing of personal data and on the free movement of that data and repealing Directive 95/46/EC (General Data Protection Regulation) (= GDPR). If research results are published, this will always be done in a blinded manner, whereby the identity of the individual test subjects cannot be traced.

A medical file will be created for you with the data that is stored about you. This file is only visible to the researchers who will conduct the studies from you. The research data from the various research centers will be stored in a central database in an encrypted form that cannot be traced back to third parties. The code table will be managed by the researcher who conducted the studies from you. You have the right to inspect the data that is stored about you, and to correct it if necessary.

Below you will find the contact details of the local administrators of the database and code table for the different centers:

**University Hospitals Leuven**
- Manager: Mr. Christophe Dausin
  Exercise Physiology Research Group
  Tervuursevest 143 - box 1505
  3001 Leuven
  Tel. +32 486 15 64 17
  e-mail: christophe.dausin@kuleuven.be

- Local data protection officer UZ Leuven: Griet Verhenneman (e-mail: gdpr.research@uzleuven.be)

**University Hospital Antwerp**
- Dr. Jessica Ratajczak
Study Coordinator / Lab Coordinator Cardiology
Cardiology, UZA
Wilrijkstraat 10
2650 Edegem
Tel. +32 3 821 40 74
e-mail: Jessica.Ratajczak@uantwerpen.be

- Local data protection officer UZ Antwerp: Filip Goyens (e-mail: DPO@uza.be)

Jessa Hospital Hasselt
- Dr. Daisy Thijs
  Jessa Hospital Hasselt
  City life 11
  3500 Hasselt
e-mail: Daisy.Thijs@jessazh.be

- Local data protection officer Jessa Hospital: Luc Ceyssens (email: dpo@jessazh.be; Tel: 011/33 50 05)

If you wish to withdraw from the study no additional data will be collected and no additional data will be transmitted to UZ Leuven.

You have the right to file a complaint about how your information is handled to the Belgian supervisory authority responsible for enforcing data protection law:

Data protection authority (GBA)
Drukpersstraat 35,
1000 Brussels
Tel. +32 2 274 48 00
e-mail: contact (at) apd-gba.be
Website: www.dataprotectionauthority.be

7. **Insurance**

In accordance with the Belgian law of 7 May 2004 concerning experiments on the human person, the sponsor is liable, even without errors, for all damage incurred by the participants and / or entitled parties that is directly or indirectly related to the study. The client of this study (UZ KULeuven) has taken out insurance that covers this liability. If you incur damage as a result of your participation in this study, this damage will therefore be compensated in accordance with the Belgian law of 7 May 2004.

Contact insurance company:
Vanbreda Risk & Benefits
Plantin and Moretuslei 297
2140 Antwerp - Belgium
Tel.: +32 3 217 67 67

8. **New information that becomes known during the research project**

During the research project, new information may become known regarding risks or benefits of the project to you. In this case we will certainly inform you. You have the right to stop your participation in this study at any time. The further policy will then proceed according to current medical practice.
9. **Results of the research project**
You will be informed of the general results of the research project. If relevant personal results are obtained, they will only be discussed with you and their impact on your further follow-up will be discussed.

10. **Ethical committee**
This study has been checked and approved by the Ethics Committee for research on subjects of the University Hospital Leuven.
If you would like further information or if you have any problems regarding the research project, you can contact the principal investigator, Prof. Dr. Contact Rik Willems or one of his employees.

11. **Reimbursement of expenses**
There are no costs for the participants in this study. The costs for all studies are borne by the study organizers.
You will be reimbursed or reimbursed during your stay in hospital on the days of the scheduled examinations.

12. **Voluntary participation in the research project**
Your participation in the research project is completely voluntary. You should not feel obliged to participate. Even if you wish to revise your consent in the course of the research, you have the freedom to withdraw from the research project. In that case, you will be asked to contact one of the employees or the principal investigator.
Thank you for reading this information brochure and for your willingness to consider participating in this study.
Consent form

Date: 26-09-2018  Place: UZ Leuven

Full title: Lifelong endurance sports for the prevention of coronary artery disease. A comparison with the late start of endurance sports training and a sedentary lifestyle.

I have read and understood the information brochure about this research project. I voluntarily decide to participate in this project under the conditions as described in the information brochure. I will receive a copy of the information brochure and of this consent form. The researchers have promised never to publicly disclose my identity and personal information.

Name of participant (in capital letters) ……………………………………………………

………………

Signature That um

I agree with a blood sample for genetic determinations that are known today or that will be developed in the future. The data about me are confidentially maintained. I can request that my DNA be removed from the analysis and destroyed at any time. The stored genetic material will only be used in the above-mentioned context.

☐ Yes  ☐ No

Signature Date

Statement from the doctor

As a researcher, I gave an oral explanation of the research brochure and answered all additional questions from the participant.

Name researcher (in capital letters) ……………………………………………………

………………

Signature That um

Name of witness (in capital letters)

………………………………………………………………………

Signature Date