RESEARCH PROTOCOL

The nature of acute functional and structural atrial changes in extreme endurance MTB cyclists taking part in the 2026 Munga as determined by contrast-enhanced cardiac magnetic resonance imaging. A pilot study.

Introduction: Background and short literature review

Mountain Biking (MTB)

Modern mountain biking (MTB) started in California in the 1970s. **(1)** MTB has grown in popularity as an outdoor recreational activity and as a competitive, Olympic, discipline. The Union Cycliste International recognizes cross-country, downhill, and stage races as distinctive events. In the 50 years since its inception MTB has undergone major sporting and technological evolution. **(2)**

Cross country MTB

Olympic cross country (XCO) has evolved since 1996, becoming 40% shorter, and both physically and technically more demanding. The discipline's physiological profile has shifted towards greater anaerobic contribution while maintaining high aerobic demands. **(2,3)** XCO circuit races have a winning time of approximately equal 120 minutes and are performed at an average heart rate close to 90% of the maximum, corresponding to 84% of maximum oxygen uptake (VO2max). **(1)** More than 80% of race time is spent above the lactate threshold. **(1)** This very high exercise intensity is related to the fast-starting phase of the race, the several climbs, forcing off-road cyclists to expend most of their effort going against gravity, greater rolling resistance, and the isometric contractions of arm and leg muscles necessary for bike handling and stabilization. **(1)** Because of the high-power output (≥500W) required during steep climbing and at the start of the race, anaerobic energy metabolism is prominent. Mountain bikers' physiological characteristics indicate that aerobic power (VO2max >70 mL/kg/min) and the ability to sustain high work rates for prolonged periods of time are prerequisites for competing at a high level in off-road cycling events. The anthropometric characteristics of mountain bikers are similar to climbers and all-terrain road cyclists. Off-road cycling economy, anaerobic power and capacity, technical ability and pre-exercise nutritional strategies play an important role. **(1)** XCO MTB consists of predominantly low velocity pedaling with a large high force component and when combined with a high oscillating work rate, necessitates high aerobic energy provision, with intermittent anaerobic contributions. Additional physical stress during downhill sections affords less recovery emphasized by physiological variables remaining high throughout. **(4)**

Multi-stage endurance mountain biking

Endurance MTB generates marked cardiovascular load, resulting in sinus tachycardia up to 91-93% of maximal heart rates. **(1,3)**

Multi-day MTB races may result in changes of heart rate variability (HRV), reflecting a decrease in vagal tone. **(5)**

The Munga

The Munga is a 1153 km, single-stage, semi-supported, MTB race over district gravel roads and remote farm tracks through the remote Karoo. **(6)**

The Munga is primarily categorized as an ultra-endurance MTB race, though it is increasingly being won on gravel bikes. The event attracts 200-250 participants, starts in Bloemfontein, finishes in Wellington, and must be completed in 120 hours. Although traditionally a MTB event, the non-technical nature of the gravel roads allows for gravel bikes. Apart from the high summer temperatures, the added 6500 meters of climbing further increases to the physical demands put on the participants. Nutritional support and mechanical assistance are provided by designated race-officials at 5 race villages, without external support allowed in between these sites. Participants carry their own supplies in-between the race villages, with 10 water points along the route.

Cardiovascular effects of endurance cycling

Endurance cyclists and similar High Static/Isometric, High Isotonic/Dynamic (Mitchell classification IIIC (HSHI)) sport disciplines like rowing and triathlon, have larger atrial and ventricular dimensions, higher left ventricular (LV) posterior wall thickness and LV mass index, as compared to endurance running, soccer, basketball, swimming, and cross-country skiing (Mitchell classification IC: Low-Moderate Static/Isometric, Moderate-High Isotonic/Dynamic demand (LMMH)). **(7,8,9)** The large resting stroke volumes and cardiac output of endurance cyclists are like those of athletes who practice LMMH disciplines. **(7,8,9)** Endurance cyclists and athletes involved in the High Static-Isotonic Mitchell class IIIC disciplines however have a higher frequency of premature atrial (PAC) and ventricular contractions (PVCs) compared with other sporting disciplines. **(7)** Endurance cyclists also have more electrocardio- graphic (ECG) abnormalities compared to any other discipline (OR 2.31 (0.90-5.88)). **(7)** The high isotonic lower body and isometric muscular upper body strain generates increased cardiac afterload, resulting in high systolic blood pressure (SBP) readings in elite cyclists, ranging 140-200 mm Hg during exercise.

Elite cyclists spend large training volumes of up to 6-7hr per day when compared to similar and other disciplines. **(7)** Longterm participants in high volume high intensity endurance sports experience more paroxysmal atrial fibrillation (PAFi) and flutter (PAFl). (**8,10**) Recreational endurance athletes including cyclist were found not to

have a higher prevalence of AFi when compared to the non-athletic population. **(11)** A recent study which evaluated senior and master recreational cyclists with ECG and cardiac ultrasound after a challenging multistage ultra-endurance cycling event, reported a marked increase in non-sustained supraventricular arrythmias without structural or functional atrial and myocardial changes. **(12)**

Atrial fibrillation and endurance sports

Moderate intensity aerobic exercise reduces the risk of cardiovascular disease including AFi (directly and indirectly), whilst vigorous lifelong endurance exercise increases the risk of AFi and atrial flutter (AFl) in male athletes by approximately 2-5-fold. **(13,14,15,16)** This higher incidence in male endurance athletes persists after retirement from active vigorous endurance exercise. **(17,18,19,20)** The benefits of moderate intensity aerobic exercise in decreasing paroxysmal AFi (PAFi) burden and boosting quality of life have resulted in its inclusion in the current PAFi management guidelines. **(19)**

A multitude of common co-morbid conditions such as central obesity, sleep disordered breathing, arterial hypertension, diabetes mellitus, and coronary atherosclerosis, and structural heart disease, have been associated with an increased risk of PAFi in the general population. **(13,14,15,19)**

PAFi is hypothesized to be the result of the presence of *substrate* (atrial fibrosis, dilation, inflammation, genetic predisposition, epicardial fat), *elicited triggers* (ectopic atrial activity, sports-nutritional supplements, illicit drugs, thyroid dysfunction, sleep disordered breathing, gastro-oesophageal acid reflux, ethanol consumption), and contributing *modulators* (autonomic modulation, increased vagal tone, sinus bradycardia, shortened atrial refractory time, electrolyte imbalances, low or high volume/intensity endurance physical activity, (exercise-induced and/or) systemic hypertension). **(13,14,15,16,19,20)** Little is however known about the etiology of PAFi and paroxysmal atrial flutter (PAFl) in endurance athletes without such known co-morbid conditions i.e. so-called ‘lone AFi/AFl’. **(16)** Risk factors for PAFi/PAFl in endurance athletes include older age, male sex, tall stature, higher volume and intensity of endurance exercise (>1778hr of self-reported life-time accumulated high-intensity endurance exercise, 3.88 [95%CI 1.55-9.73] fold higher risk of lone PAFi), elite level endurance athletics, longer competitive career, atrial dilation, ‘high-static/high-dynamic sport disciplines’, specifically cycling, rowing and long-distance cross-country ski-racing. **(10,23,24,25,26,27,28,29)** However, increasing lifetime training volume in recreational athletes with resulting atrial dilation, was not associated with increased vagal parameters or atrial ectopy, established risk factors

of PAFi. In some studies exercise-induced hypertension (systolic blood pressure (SBP) >210mm Hg in males, >190mm Hg in females, diastolic blood pressures (DBP) >110 mm Hg) was foundto be a risk factor for PAFi. **(25,26)**

Female endurance athletes

Female endurance athletes have been under-represented in studies which revealed conflicting data concerning the prevalence of PAFi/PAFI. **(21,23,33,34,35,36,37,38)** Compared to the general, non-athletic population PAFi /PAFI seems more prevalent in female endurance athletes, but the absolute rate of AFi/AFI in female athletes may be lower when compared to male endurance athletes. **(21,23,33,34,35,36,37,38)** The studies which compared female to male athletes have included relatively younger females with lower life-time accumulated endurance exercise volumes and smaller atria, which prevented matched comparison. **(33,35,38)** Both sexes had similar rates of atrial and ventricular ectopy, similar amounts of magnetic resonance quantified myocardial fibrosis, but female athletes had less bradycardia, non-sustained ventricular tachycardia (nsVT) and PAFi, lower resting and exercise induced systemic blood pressures, less myocyte hypertrophy, and smaller cardiac compartments. **(38)**

Genetics of Sinus Bradycardia, Atrio-Ventricular Conduction Delay and PAFi in endurance athletes

In endurance athletes and the general non-athletic population, a non-specific inherited polygenic risk, related to over a hundred genetic loci which encode ion channels, transcription factors and post-transcriptional regulatory mechanisms, seems to modify the risk for PAFi in the presence of atrial substrate. **(14,39,40)** Sinus bradycardia (SB) and intermittent atrio-ventricular conduction delay or conduction block (AVB) in endurance athletes have been demonstrated to be benign in nature and related to genetic predisposition. **(41,42)** The potentially greater athletic endurance performance resulting from SB, resulting higher heart rate reserve (HRR) and peak cardiac output may thus be partly genetically predisposed. **(41,42)**

Animal models of exercise induced atrial inflammation, fibrosis, and fibrillation.

Small human studies have reported atrial histopathological findings of lone atrial fibrillation in non-athletic populations. Changes included interstitial atrial fibrosis, increased intercellular space, myofibrillar loss, decreased nuclear density, and lympho-mononuclear cell infiltrates suggestive of isolated atrial inflammation. **(43,44,45,46,47)** A recent small pilot study which evaluated highly trained Caucasian

master and veteran cyclists with and without documented PAFi after a bout of intense exercise, demonstrated novel biomarkers associated with pro-inflammatory and fibrotic pathways associated with exercise-induced atrial inflammation, fibrosis, and atrial arrhythmias. **(48)** In the absence of histopathological data in athletes, small animal models have provided direct and indirect histological evidence for the development of high-intensity endurance exercise induced (reversible) atrial fibrosis and resulting atrial arrhythmias. **(49,50,51,52,53)**

Ultrasound assessment of the acute and chronic atrial adaptations in endurance exercise

A large number of studies employing cardiac ultrasound have detailed the acute and chronic (patho-)physiological changes in recreational and elite endurance athletes. A small study evaluating the acute LA adaptations in male elite mountain marathon runners demonstrated a transient increase in markers of acute inflammation, and increased LA wall tension without dilation. **(56)** This may reflect exercise-induced LA myocardial oedema and contribute to long-term atrial remodelling, generating an eventual substrate for atrial arrhythmias. **(56)** Ultrasound is however unable to differentiate between atrial oedema, stunning or fibrosis. A study evaluating trail runners demonstrated a dose-dependent relationship between the progressive decrease of atrial strain with increasing race distance. **(57)** Peak right and left atrial reservoir and contraction strain decrease with increasing acute and chronic ('lifetime') endurance volume and intensity ('load'), but more so in male compared to female endurance athletes. **(58,59)** Atrial dilation, and the decrease in LA systolic atrial function was more pronounced in male compared to female elite endurance athletes and may signify early structural and electrophysiological changes which predispose to PAFi. **(61)** With accumulating lifetime volumes of intense endurance exercise both atrial and ventricular volumes expand to the same extend. **(58,60)** Atrial volume positively correlates with lean body mass, maximal oxygen uptake (VO2max) during cardio-pulmonary testing, and lifetime cumulative volume of vigorous endurance exercise in both sexes, and all studied ethnicities. **(61)** During prolonged vigorous endurance exercise atrial longitudinal strains (conduit, reservoir, contractile) decrease, presumably due to inflammation and fibrosis. **(59,62)** Gan et al. Whilst LV volumes and mass reverse with retirement from competitive endurance sports, permanent structural and electrophysiological substrate of the irreversibly dilated LA result in persistent higher rates of PAFi after years of detraining. **(18)** Individuals with PAFi have larger atria, impaired reservoir and contractile strain, with

an existing overlap in atrial volumes and strain between endurance athletes and the non-athletic population with PAFi. **(28,63)** Exercise echocardiography demonstrated impaired atrial contraction reserve in a subset of endurance athletes, which may signify atrial inflammation, fibrosis, early atrial dysfunction, potentially resulting in impaired exercise performance. **(64)** Left atrioventricular coupling (LAVC) in master endurance athletes relates to the degree and manner in which the LA and LV mechanical functions are synchronized. Impaired LAVC is a predictor of PAFi, Heart Failure with preserved Ejection Fraction (HFpEF) and is influenced by age, training history, sport discipline, co-morbidities such as HPT, as well as the resulting structural and electrical atrial and ventricular adaptations. **(65)** LA remodelling in master endurance athletes results in LA dilation (LAD), decreased compliance i.e. increased stiffness, resulting in loss of LA conduit ('propulsion') and reservoir ('storage') functions. Atrial remodelling is the result of a complex interplay of physiological age related, and exercise related atrial, ventricular, and vascular changes. Resulting changes in LV filling which affect endurance performance. **(66)**

Left atrial fibrosis and inflammation demonstrated by *CMR in endurance athletes*

Contrast-enhanced magnetic resonance (CMR) has been widely used in sports medicine to evaluate myocardial mass, ventricular functions, inflammation and interstitial or replacement fibrosis. CMR has demonstrated reversible right ventricular (RV) and LV myocardial oedema, as well as impaired myocardial longitudinal strain after intense endurance exercise in cyclists and triathletes. **(67,68,69)**

Though technically demanding, LA late gadolinium-enhanced CMR (LA LGE CMR) was introduced in clinical practise in 2014 and was mainly employed to evaluate volumes, functions, strain, inflammation and fibrosis in the non-athletic population with PAFi. **(70)** In the general non-athletic aging population LA volume and the presence and extent of LA late gadolinium enhancement (LGE) with cardiac magnetic resonance (CMR) predict the onset and burden of PAFi. **(70,71)** Three CMR studies have sofar evaluated endurance athletes for LA fibrosis. Peritz evaluated a young athletic population and used a controversial method quantifying LA LGE, while Javed included master athletes with PAFi. We recently evaluated 42 middle-aged life-time Master endurance athletes (76% male, age 54±9yr) and demonstrated >5% LA LGE (threshold for abnormal) in 13 (31%). **(31,54,55)** The median fibrotic burden was 2.5% of LA surface area (IQR 1.1-7.6%). Fibrosis was heterogeneously distributed across the LA, with the highest burden involving the posterolateral segments. The LA fibrotic burden was significantly higher in the cross-country MTB cyclists compared to road runners (7.3% [4.1-9.5] vs 2% [0.5-5.6],

p=0.03). The presence and extent of LA LGE may predispose these athletes to future PAFi. **(31)**

author	**athletic population** (number)	**LAVImax (ml/m^2)** (≤50)	**LAEF (%)** (57±13) (31-83)	**LA LGE (%)** (analysis) (<5%)
Peritz **(54)**	n=20SR, 48.7±8yr, 75%M, 100%C	40.6±8.7	?	15.5±5.9 (MARREK)
Hopman **(55)**	n=42SR, 54±9yr, 74%M, 100%C	46.1±14.6	60.0±7.1	1.1-7.6 (ADAS 3D)
Javed **(31)**	n=39PAFi, 63.0±6.2yr n=94SR, 58.7±5.4yr, 100%M, 100%C	PAFi 54.7±17.8 SR 53.0±12.1	PAFi 52.1±11.6 SR 57.2±7.8	PAFi 4.7-9.2 (ADAS 3D) SR 3.8-11.4 (ADAS 3D)

[C=Caucasian, M=male, PAFi=paroxysmal AF, SR=sinus rhythm]

For the first time we were recently also able to demonstrate the potential reversal nature of extensive LA LGE in a competitive high-volume Master MTB cyclist after detraining. (**Figure 1,** submitted for publication) Our data suggest that in some athletes LA LGE may represent atrial inflammation, a novel finding. LA LGE CMR studies to evaluate for acute inflammatory atrial changes after extreme endurance exercise have not yet been performed. **Figures 2** and **3** represent the findings in respectively a life-long high volume/low intensity 62yr ultra-road runner (estimated accumulated life-time distance 50 000km/2500 hours) and 53yr recreational male moderate volume/intensity endurance trail runner with PAFi.

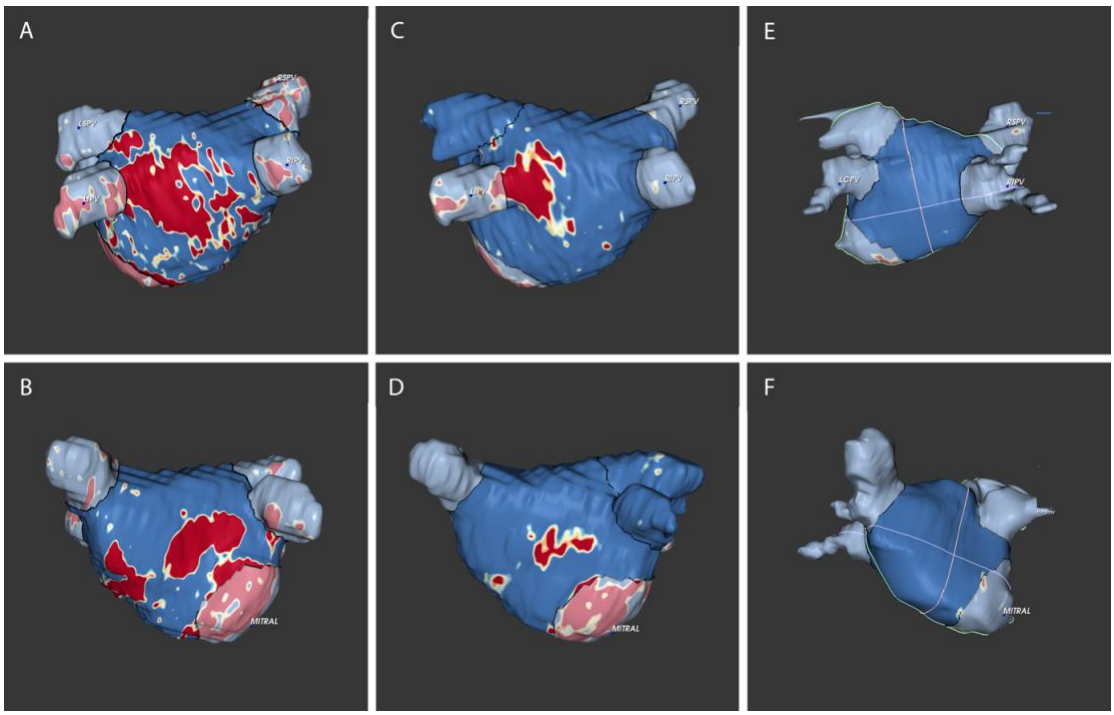


Figure 1: Demonstration of reversible LA inflammation in a 57yr high volume/ high intensity endurance MTB cyclist who had intensively exercised for 20yr. After he radically decreased his endurance load the extent of LALGE resolved over the course of two years. **1A:** Baseline 3D LA LGE posterior-anterior (PA) view, blue-unenhanced atrial wall, red-late gadolinium enhanced. The CMR protocol and analysis have been previously reported (Hopman et al, 2025);**1B**: *Baseline* 3D LA LGE anterior-posterior (AP) view. **1C:** *21 months* FU: 3D LA LGE FU (PA view), **1D:** *21 months* FU: 3D LA LGE FU (AP view). **1E:** *24 months* FU: 3D LA LGE FU (PA view), **1F:** *24 months* FU: 3D LA LGE FU (AP view).

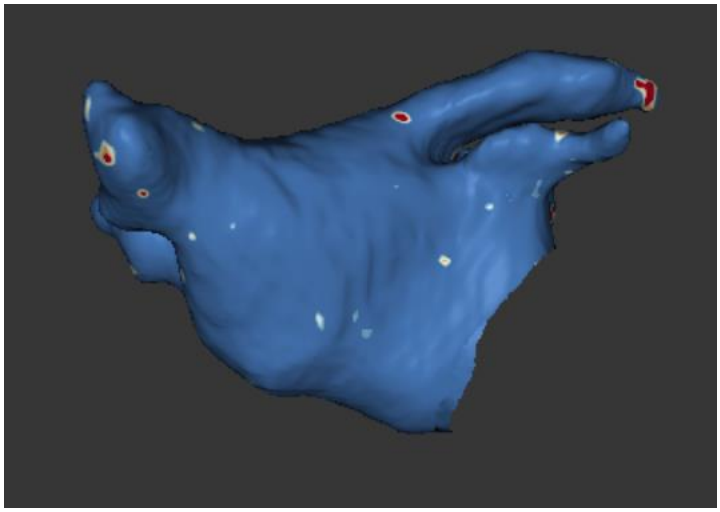


Figure 2: Example of LA LGE CMR study of a 60yr lifetime high volume/low intensity ultrarunner demonstrates the absence of fibrosis.

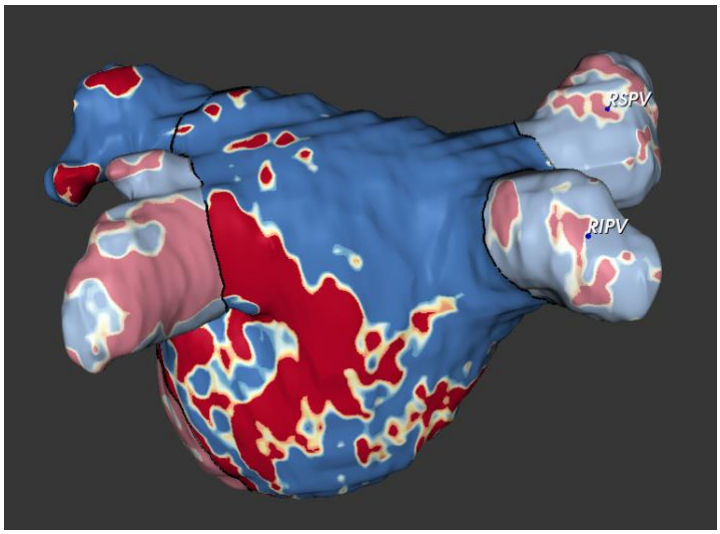


Figure 3: A 53yr recreational male endurance athlete with progressive exercise related PAFi, moderate LA dilation (121 ml, 58 ml/m^2), impaired LAEF (35%), impaired reservoir strain (17%), and extensive LA LGE (30%). Multimodality evaluation has confirmation the relationship between LA fibrosis, impaired LAEF, reservoir strain and risk of PAFi. **(73)**

Atrial Cardio Myopathy and endurance exercise

Some lifelong high volume/high intensity Master endurance athletes may develop atrial myopathy (AtCM), broadly defined as 'any complex of structural, architectural, contractile or electrophysiological changes affecting the atria, with the potential of creating clinical manifestations'. **(40,74,75)**

AtCM is reflected by larger atrial volumes, lower atrioventricular volume ratios, mildly reduced LA longitudinal strain, mild atrial fibrosis, longer P wave duration on the 12-lead ECG, greater atrial ectopy, and eventually PAFi. PAFi itself may promote the development of AtCM. **(76)** Trivedi et al studied athletes and non-athletes with PAFi and revealed different functional associations between the two groups, suggestive of different pathophysiological mechanisms. Diastolic dysfunction and reduced strain defined non-athletes with AF. Athletes had low atrial strain and those with AF had enlarged LA volumes and reduced atrial emptying but preserved LV diastolic parameters. Thus, AF in athletes may be triggered by an atrial myopathy from exercise-induced haemodynamic stretch consequent to increased cardiac output. **(77)** The structural and functional changes of atrial tissue may be clinically silent or manifest as PAFi, congestive heart failure (CCF) and cardioembolic stroke (CE-CVA) (**75)** Male sex, age >65yr, coronary artery disease (CAD), arterial hypertension (HPT), obesity, and high alcohol intake >15 units/week are risk factors for AtCM. **(74,75,76)**

The relevance of serum iron for endurance exercise

Iron is a crucial element in the human body, central to providing red cell oxygen carrying ability, mitochondrial cytochrome, myoglobin, skeletal and myocardial functions, body temperature regulation, and to cerebral and peripheral nerve conduction processes. Iron deficiency impairs aerobic cardiovascular performance (VO2max), endurance, muscle strength and slows down physical recovery after physical exertion. **(78)** Iron deficiency is common in Sub Saharan Africa, particularly in females of childbearing ages, though reliable statistics are not available. **(79,80)** Endurance athletes lose more iron due to sweating, red cell breakdown due to foot strike, and gastrointestinal loss. **(78)** Iron is an important micronutrient in pathways of energy production; adequate nutrient intake and its balance is essential for optimal athletic performance. However, large studies elucidating the impact of iron deficiency on athletes' performance are sparse. Recently Keller et al demonstrated that iron deficiency was independently associated with reduced VO2max in competitive athletes of any age. **(81)** Iron deficiency occurred more frequently in female athletes

and decreased with aging. **(81)** Oral iron supplementation improves the iron status and aerobic capacity of iron deficient non-anaemic endurance athletes. **(82,83)**

Knowledge gaps

The mechanisms of PAFi in otherwise healthy athletes ('lone atrial fibrillation') is poorly understood. Female and non-European athletes have been under-represented in studies. The research data concerning the relationship between increasing life-time cumulative (high-intensity) endurance volume, atrial volumes, age and hormonal status in females are incomplete and sometimes conflicting. There is a scarcity of human data concerning the role of atrial inflammation and fibrosis in endurance athletes. **(84,85)** Contrast-enhanced CMR is the only imaging modality with the spatial resolution and capabilities to evaluate atrial volumes, systolic function, longitudinal strain, inflammation and fibrosis. **(31,54,55)**

Problem statement

Long-term excessive, vigorous endurance exercise may cause pathologic structural remodeling of the heart in predisposed individuals. **(9,10,18,20,23)** Data suggest that chronic training for and competing in extreme endurance events may cause acute volume overload of the atria, with transient inflammation, resulting reductions in atrial reservoir and contractile functions, and elevations of cardiac biomarkers, all of which return to normal within 1 week of finishing. **(17)** Master endurance athletes have a reported high incidence of atrial arrhythmias. **(9,17,18,20,23)** The acute atrial adaptations in strain, volume, systolic function, and presence of atrial inflammation after an acute bout of endurance cycling have never been investigated with advanced LGE CMR.

South Africa is home to many (ultra-)endurance athletes, but little research has evaluated acute or chronic cardiovascular (mal)adaptations in our endurance athletes. A single small South African study evaluated acute ventricular adaptations with 2D cardiac ultrasound in a small number of male Comrades participants and reported post-race increase of RV and LV electromechanical delay with mildly reduced LV systolic function. Little is known about the acute atrial adaptations in endurance MTB athletes. Our study addresses this current gap in knowledge concerning the acute atrial adaptations in South African ultra endurance cyclists.

Research questions and hypothesis

Therefore, the aim of this observational pilot-study is to assess the morphological and functional atrial characteristics of a cohort of individuals who take part in the 2026 Munga Mountain Bike Race (MTB) (25-30th November 2026) employing state-of-the-art magnetic resonance imaging technology. Current ultrasound technology allows for robust semi-automated three-dimensional delineation and quantification of the atrial volumes and acute changes thereof. Two-dimensional longitudinal strain quantification allows for superior detection and quantification of changes in segmental and global systolic atrial. Previous studies have reported variable and sometimes conflicting findings in endurance athletes after ultra-endurance races. We expect current magnetic resonance technology to demonstrate physiological changes in the atrial dynamics after Munga MTB, which will allow us greater understanding of the stress of this type of exercise on the athlete's heart. We hypothesize acute increases in bi-atrial volumes, decrease in strain and systolic atrial functions, with accompanying increase of atrial LGE. **(45)**

Study objectives:

To determine the acute adaptive atrial changes in endurance MTB cyclists during the 2026 Munga MTB with advanced contrast-enhanced CMR:

1.acute three-dimensional (3D) LA changes in volume, ejection fraction, as well as two-dimensional (2D) LA longitudinal strain.

2.to determine the presence of biatrial LGE in life-long endurance cyclists before taking part in the 2026 Munga. (baseline LGE: chronic atrial inflammation, fibrosis)

3.to determine and quantify any increase of biatrial LGE in the identical athletes after finishing the 2026 Munga. (post-race acute change in LGE: atrial inflammation)

4.to determine the relationship between the athlete's accumulative lifetime volume and intensity of endurance exercise and the measured structural and functional atrial parameters at baseline.

Research Design, Methods and Participants

The Munga Heart Study is an open, purely observational pilot study. Annually approximately 200-250 endurance MTB cyclist enrol and start Munga. Depending on weather conditions the historic attrition rate of the Munga MTB generally fluctuates between 20-45%. The Munga Heart Study aims to enrol 26 participants. Permission to undertake this study has already been formally granted by the organisers of the 2026 Munga (25th-30th November 2026) (*Appendix 5)* Twenty-six (26) healthy male (13) and female (13) cyclists will be recruited by advertising our study through The

Munga website, internet forums and social media platforms via recruitment material (*Appendix 1*). The aim will be to have the number of participants evenly divided between the two sexes (13 female, and 13 male athletes) and include cyclists with different levels of experience, physical fitness and anticipated finishing times. Based on published statistics of previous Munga endurance races, we anticipate 30% (9 athletes) not to finish the race, thus expecting a total of 17 finishers.

Inclusion criteria

- Male and female Munga participants aged 35-70 years of age

Exclusion Criteria

- Existing known cardiovascular pathology such as intermittent supra-ventricular or ventricular arrhythmias, atrioventricular or intraventricular conduction disease, arterial hypertension, cardiomyopathies, valvular heart disease, and coronary artery disease, which would affect the structural or functional activity of the heart and the results of the assessment.
- Inability or unwillingness to provide written consent.

All clinical investigations will be conducted according to the principles expressed in the Declaration of Helsinki (2013). Participants will provide written informed consent to have the cardiac ultrasound assessment performed before inclusion in the study. Participants may withdraw from the study at any time.

Study protocol: research procedures

1.Initial interview

Participants will complete a telephonic interview, and/or emailed questionnaire concerning their medical and athletic history and will be informed in full about the nature of the research study and cardiac assessments. Digital and/or written information documents will be provided *(Appendix 2)*.

Any questions related to the study will be answered and when willing, the subject will sign the consent form *(Appendix 3)*. Following enrolment, the participant will undergo cardiac assessment (history, physical assessment, basic laboratory assessment, body composition assessment, resting electrocardiography, resting and exercise echocardiography, bicycle VO2 max test, and contrast-enhanced cardiac magnetic resonance) before and follow up contrast-enhanced cardiac magnetic resonance within three days after finishing the race.

2.Resting 12-lead electrocardiography

In the 3 weeks before The Munga study participants will be scheduled to have a resting 12-lead electrocardiogram (ECG) and ultra-high frequency electrocardiogram (UHF-

ECG). A second UHF-ECG will be performed in the Race Village immediately after finishing the race to detect and quantify any electrical ultra-endurance exercise induced interventricular and intraventricular conduction delays**. (86)** The supine resting 12-lead ECG will be performed and interpreted according to the generally applied Updated 2026 International Criteria. **(87)** Standard commercially available equipment will be used. The ECG data of the athletes will be deidentified – each individual athlete will be coded with a number (1-26, subsequent numbers as participant are enrolled). The athlete's personal data, and health information linked to this number will be kept separately on a password protected laptop kept in the offices of dr Smedema. The laptop computer is password protected, and only accessible by the principal investigator and co-investigator. Data will be backed up an external hard drive and kept locked in the offices of the co-investigator. The ECG system with the deidentified studies will be kept in the offices of the co-investigator (Sports Cardiovascular Research Program (CRP), Netcare Blaauwberg Hospital, room 2C5, Waterville Crescent, Sunningdale 7441, tel 021-5543731). These premises are covered by 24-hours security.

3.Baseline and follow up resting transthoracic echocardiography

Before cycling The Munga all participants will be studied with the Philips EPIQ CVxi dedicated cardiac ultrasound system (Philips, Best, The Netherlands) at Netcare Blaauwberg Hospital room 2C5 - CRP. The evaluation will be performed according to International Guidelines and include the standard 2D and 3D measurements of compartmental volumes, myocardial mass, and myocardial functions. Auto Measure AI provides fully automated 2D Doppler and length measurements. AutoStrain delivers fast, reproducible 2D strain quantification for the LV, LA and RV. By manually delineating the RA, off-label use of AutoStrain will allow for strain assessment of this structure. Dynamic HeartModel and 3D Auto RV offers full 3D quantification for LV, LA and RV volumes functional assessment. Left and right atrial strain, booster pump, reservoir and conduit functions will be determined. **(31)** Tissue Doppler, continuous wave and pulsed wave Doppler will be used to estimate pulmonary pressures and left ventricular filling pressures. A full resting cardiac ultrasound study will take an estimated 8-12 minutes. The athletes will be positioned on the left side with legs raised to optimize and standardize cardiac preload. Within 1 hour of finishing the race athletes will be subjected to an identical second cardiac ultrasound study (10 minutes) in the race village in Wellington.

4.Bicycle exercise echocardiography

Following the resting assessment athletes will be subjected to bicycle exercise electro/echocardiography to exclude inducible myocardial ischemia and evaluate left atrial contractile reserve. **(64,88)** The generated data of the athletes will be deidentified – each individual athlete will be coded with a number (1-26, subsequent numbers as participant are enrolled). The athlete's personal data, and health information linked to this number will be kept separately on a password protected laptop kept in the offices of dr Smedema. The laptop computer is password protected, and only accessible by the principal investigator and co-investigator. Data will be backed up an external hard drive and kept locked in the offices of the co-investigator. The ultrasound data with the deidentified studies will be kept in the offices of the co-investigator (Sports Cardiovascular Research Program, Netcare Blaauwberg Hospital, room 2C5, Waterville Crescent, Sunningdale 7441, tel 021-5543731). These premises are covered by 24-hours security.

5.Cardio-Pumonary Exercise Testing

To determine their baseline level of aerobic fitness maximal oxygen uptake during peak exercise (VO2 max) Munga study participants will be scheduled to have a bicycle cardio-pulmonary exercise test in the 3 weeks before the race. The generated data of the athletes will be deidentified – each individual athlete will be coded with a number (1-26, subsequent numbers as participant are enrolled). The athlete's personal data, and health information linked to this number will be kept separately on a password protected laptop kept in the offices of dr Smedema. The laptop computer is password protected, and only accessible by the principal investigator and co-investigator. Data will be backed up an external hard drive and kept locked in the offices of the co-investigator. The CPET system with the deidentified studies will be kept in the offices of the co-investigator (Sports Cardiovascular Research Program, Netcare Blaauwberg Hospital, room 2C5, Waterville Crescent, Sunningdale 7441, tel 021-5543731). These premises are covered by 24-hours security.

6.*Evaluation of sleep quality using wearables*

Athletes who monitor their sleep quality with a wearable device in the week leading up to The Munga will be asked to share their data.

7.Heart rate monitoring during Munga

Athletes who have completed the race whilst monitoring their heart rate with a wearable device will be asked to share their heart rate data as to determine the

intensity of their efforts. The percentage race time in the different individual heart rate zones, as determined by the athletes age will be determined.

8.Bioelectrical Impedance Analysis

All participants will be studied with the InBody S10 (InBodySA, Pty Limited) in the week before and when finishing the race to determine the total amount and the distribution of body fat (limbs, trunk, visceral), muscle (limbs, trunk), water (intracellular, extracellular) as well as cellular integrity of skeletal muscle. Athletes will be assessed in supine position, bare feet, the arms and torso, and legs and thighs not touching. This equipment is commercially available, well validated and internationally widely used. **(89)**

9.Contrast-enhanced Cardiac Magnetic Resonance Imaging

Contrast-enhanced CMR will be scheduled in the 2 weeks before and the 3 days after finishing The Munga. CMR will be performed according to our previously published protocol and will be limited to structural and functional atrial evaluation. ***(55)*** All scans will be performed using a 1.5 Tesla clinical MRI system (Siemens, Erlangen, Germany) at Cape Radiology, Constantiaberg Medi-Clinic, Plumstead, Cape Town. High resolution 3D atrial LGE images will be acquired using a navigator-based respiration and ECG-gated inversion recovery prepared gradient echo pulse sequence applied approximately 13-17 minutes after administration of Gd-DO3A-butrol – gadobutrol (Gadovist®) at a rate of 2.5ml/s. Depending on the respiratory pattern and the heart rate of the participant, acquisition of the 3D LGE images will take approximately 10-15 minutes. Volumetric measurements for the atria will be obtained from two-chamber and four-chamber cine images using the biplanar method. Atrial volume and global function assessment as well as longitudinal strain analysis will be conducted using Circle CVI (Version 5.11, Circle Cardiovascular Imaging, Inc, Calgary, Canada). Fibrosis assessment will be performed using ADAS 3D LA (Adas3D Medical SL, Barcelona, Spain).

Ethical considerations

Participants will only be enrolled into the study once the Human Research Ethics Committee has approved the protocol. Subjects will be fully informed as to the nature the study and will sign informed consent prior to participation. All procedures will conform to the 2013 Declaration of Helsinki. The investigators maintain responsibility for informing the Human Research Ethics committee in writing of any amendments to the protocol or of any adverse events resulting from participation in the study.

Risks to participants

This study is purely observational and descriptive in nature and concerns the use of cardiac exercise testing and innocuous, non-invasive, ultrasound and magnetic resonance imaging technology. There may be injury risk when performing exercise during the exercise echocardiography and VO2max assessment, like the risks an athlete would be exposed to when training or racing. Medical risk will be minimized as there will be a cardiologist certified in cardiopulmonary resuscitation present at all exercise sessions. Participants are not subjected to any procedural risk. All ultrasound scanning will be conducted by Dr JP Smedema, a specialist cardiologist in private practice and registered with the HPCSA. Should any cardiac abnormalities be noted in the initial ultrasound scan, Dr Smedema will apply the well accepted Bethesda Guidelines with respect to athletes' ability to participate in the sport. In the unlikely case of the diagnosis of underlying cardiac pathology by ultrasound, Dr Smedema will advise the athlete regarding participation and will refer the patient to their own existing medical practitioner or to a state health facility for further work-up. During CMR screening the participants will be injected with a contrast medium. The risk of adverse events relating to gadolinium-based contrast agents (GBCA) carries a very low rate of adverse events (0.01-2.4%), lower compared to low osmolar non-ionic iodinated contrast agents for computed tomography. **(90-94)** The most relevant safety issue is its restricted use to patients with preserved renal function. Nephrogenic systemic fibrosis (NSF) has been reported in a small subgroup of patients with an estimated glomerular filtration rate < 30 ml/min/1.73 m^2. The FDA has published guidelines for the use of GBCA in this population. **(94)** All the study participants will have their renal functions assessed and those with an estimated glomerular filtration rate < 30 ml/min/1.73 m^2 will be excluded. This will be assessed by means of a biochemical blood test which will be collected under sterile conditions by a trained phlebotomist.

Benefits to participants

Each participant will be given unique medical insight into the health and functional adaptation of their hearts and any risks that their exposure to high volume training in the past may have caused. They will be provided with best-in-class medical advice regarding the management of any pathology if this is identified.

Data safety and management

Personal data of the athletes will be deidentified – each individual athlete will be coded with a number (1-26, subsequent numbers as participant are enrolled) actual imaging

data on the ECG, CPET and ultrasound systems will be stored under this number, the athletes personal data, health information and acquired ECG, exercise and cardiac ultrasound measurements linked to this number will be kept separately on a password protected laptop kept in the offices of dr Smedema. The laptop computer, which is password protected, and only accessible by the principal investigator and co-investigator. Data will be backed up an external hard drive and kept locked in the offices of the co-investigator. The ECG and ultrasound system with the deidentified cardiac imaging studies will be kept in the offices of the co-investigator (Sports Cardiovascular Research Program, Netcare Blaauwberg Hospital, room 2C5, Waterville Crescent, Sunningdale 7441, tel 021-5543731). Imaging studies will be transferred to a password protected off-line desktop-based computer system with advanced post-processing software. This system is only accessible by the co-investigator. These premises are covered by 24-hours security.

Time frame

Telephonic and on-line, web-based engagement with athletes will take place in the weeks before race day, the 25th of November 2026.

Baseline cardiac assessment will take place in the days-week before the race. Follow up CMR assessment of athletes who have successfully finished Munga will take place in the three days after finishing the race.

Reporting of results

The acquired data will be analysed during the 4 weeks after the 2026 Munga MTB. After final analysis results will be shared with the participants, presented at one local conference meeting, and submitted for publication in an international peer reviewed professional journal. The current study will provide us with information on the prevalence, nature, extent and distribution of acute functional atrial changes in endurance cyclists. It is anticipated that the findings of our current project will lead to more detailed follow up.

Significance of the study

To date there have been no studies utilising advanced magnetic resonance to assess acute structural and functional atrial changes in a cohort of endurance MTB athletes exposed to a habitually high exercise load. This study will provide unique insights that should identify key features for more targeted future studies.

Appendix 1 *Advertisement for Recruitment*

The 2026 Munga Heart Study

Dear Athlete,

Although regular exercise is healthy and is associated with a lower risk of developing chronic diseases and a lower risk of heart disease and death, excessive exercise may pose a risk to your health.

We are conducting a research study to assess the effects of long-distance off-road cycling on the structure and function of the heart.

This study will take place in the weeks before the 2026 Munga MTB and within the three days after finishing, where you would be required to have a second cardiac magnetic resonance study (duration 30 minutes).

If you are between the age of 35 and 70 and have successfully entered the 2026 Munga MTB then you may be suitable to participate in this study.

If you are interested, please contact us at the details below:

Dr Jan-Peter Smedema
Tel: 0720410379
Email: jansmedema@hotmail.com

Appendix 2

PARTICIPANT INFORMATION SHEET AND CONSENT FORM
The Munga Heart Study (25th -30th November 2026)

Investigator: Dr Jan-Peter Smedema
Tel: 0720410379
Email: jansmedema@hotmail.com

Dear Participant

We would like to invite you to take part in a research project which will be conducted by the Sports Cardiology Research, at Netcare Blaauwberg Hospital, Sunningdale, and Cape Radiology, Plumstead, Cape Town, to determine the acute effects of prolonged intense off-road cycling on the structure and function of the heart. The novel information obtained from this study will be used to plan future research studies which will focus on the long-term benefits and risks of intense endurance exercise such as ultra-distance cycling.

This study will be supervised by Dr Jan-Peter Smedema from the Sports Cardiology Research Program, and professor Vernon Louw of University of Stellenbosch.

Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are completely satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is entirely voluntary, and you are free to decline to participate. In other words, you may choose to take part, or you may choose not to take part. Nothing bad will come of it if you say no: it will not affect you negatively in any way whatsoever. Refusal to participate will involve no penalty or loss of benefits or reduction in the level of care to which you are otherwise entitled. You are also free to withdraw from the study at any point, even if you do agree to take part initially. The Health Research Ethics Committee approved this study. The study will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, the South African Guidelines for Good Clinical Practice (2006), the Medical Research Council (MRC) Ethical Guidelines for Research (2002), and the Department of Health Ethics in Health Research: Principles, Processes and Studies (2015).

Why is this study being done?

International studies have shown that veteran endurance athletes have a 2-5-fold increased risk of ‘atrial fibrillation’, an arrhythmia which generally results in a fast, irregular pulse rate, and is associated with an increased risk of stroke. This risk appears related to scarring of the top compartment (‘atria’) of their hearts when

evaluated with cardiac magnetic resonance/MRI. Some preliminary research suggests that these scars may be the result of persistent vigorous endurance exercise like running and cycling. Our current study will help us describe the acute effects of prolonged, vigorous exercise on the heart in ultra-endurance cyclists with novel ultrasound and magnetic resonance technology. Our findings will be used to plan more detailed studies which will evaluate the effects of endurance cycling over years.

What will happen if you decide to participate in the study? You will be contacted by telephone or email and asked to provide information concerning your medical and athletic history.

During the first assessment

You will have basic laboratory assessment (electrolytes, renal functions, haemoglobin, iron studies), InBody-body composition assessment (5mins), a regular resting and ultra-high frequency electrocardiogram (ECG, 5mins), undergo resting and bicycle exercise echocardiographic (ultrasound of your heart, 30 min) assessment, and a cardio-pulmonary exercise test (VO2max, 30 min) during the 21day(s) before the 2026 Munga race (25^{th}-30^{th} November 2026) in Cape Town at Netcare Blaauwberg Hospital – Room 2C5 – Sports Cardiovascular Research Program (Waterville Crescent, Sunningdale 7441, Cape Town). During the 2 weeks before Munga you will also be invited to have a and limited MRI of your heart at Cape Radiology, Constantiaberg Medi-Clinic, Plumstead (30 min). Your height, weight, circumferences of neck/waist/hips, skin fold thicknesses and resting blood pressure readings will be determined at the same time. During the ultrasound study you will be asked to turn on your left side and hold your breath intermittently while the sonographer will gently hold a small ultrasound probe or camera against your chest to obtain images of your heart. In the week preceding the race we'll ask the athletes who have access to wearables to access sleep data. Athletes who aim to wear a heart rate monitoring device during the race will be asked to record and store the acquired heart rate data. Dr Smedema is a very experienced cardiologist and will be able to provide you with advice regarding any findings that may present during the ultrasound scans. In the very unlikely circumstance that our assessment reveals abnormalities which could be potentially detrimental to your health whilst taking part in this year's race, we may advise you not to take part in the 2026 Munga MTB and refer you to the appropriate healthcare provider.

During the second assessment – after finishing in the Race Village (Wellington),

you will have a repeat electrocardiogram (ECG) performed at the race village, followed with the second cardiac MRI study at Cape Radiology, Constantiaberg Medi-Clinic, Plumstead (30 min) during the 3 days after finishing Munga. Athletes who acquired heart rate data during the race will be asked to share their data with the researchers.

What are the risks of this study?

There are no risks associated with this study. Assessment with electrocardiography, ultrasound, bio-impedance analysis (InBody), and MRI is harmless. Your personal data will be protected – each individual athlete will be identified with a number, imaging data on the ultrasound system will be stored under this number, the athlete's personal data linked to this number will be kept separately on a password protected laptop kept in the offices of dr Smedema which enjoy 24-hour security protection.

Will your results be shared with you?

Yes, you will be given feedback on the findings (a formal printed ultrasound report will be provided) and we will discuss any medical implications with you and how to manage these. Dr Smedema is a very experienced cardiologist and will be able to provide you with advice regarding any findings that may present during the ultrasound scans.

Will you receive any reward for taking part in this study?

No financial compensation will be available for participation in this study.

If you have any more questions regarding this study please feel free to contact any individuals listed below.

You can contact Dr Smedema at 0720410379 if you have any further queries or encounter any problems.

You can contact the Health Research Ethics Committee if there still is something that your study doctor has not explained to you, or if you have a complaint.

You will receive a copy of this information and consent form for you to keep safe.

Dr Jan-Peter Smedema

Appendix 3

Consent form

The 2026 Munga Heart Study

By signing this document below, it serves as confirmation that you have read through the study information sheet and that you have understood the information sheet and consent form and that you are willing to participate in this study. You have the right to withdraw at any time, and you may ask questions at any time during the study. All information recorded in this study will remain confidential and no participants will be identified in the event of future publication

Declaration by participant

By signing below, I ……..……. agree to take part in a research study entitled "The prevalence of acute functional atrial changes in solo-riders/participants of the 2026 Munga MTB (25-30 November 2026) as determined by advanced cardiac MRI. A pilot study."

I declare that:

- I have read this information and consent form, or it was read to me, and it is written in a language in which I am fluent and with which I am comfortable.

- I have had a chance to ask questions, and I am satisfied that all my questions have been answered.

- I understand that taking part in this study is voluntary, and I have not been pressurised to take part.

- I may choose to leave the study at any time and nothing bad will come of it – I will not be penalised or prejudiced in any way.

- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan that we have agreed on.

Signature of participant

Signed at (place) .....…..…… on (date) ……...…….. 2026.

Participant Name: _____

Date _____

Witness signature _____

Witness Name: _____

Date _____

Declaration by investigator

I (name) ……..…… declare that

- I explained the information in this document in a simple and clear manner to ……..
- I encouraged him/her to ask questions and took enough time to answer them.
- I am satisfied that he/she completely understands all aspects of the research, as discussed above.
- I did/did not use an interpreter. (If an interpreter is used then the interpreter must sign the declaration below.)

Signed at (place) .....…..……. on (date) ……...…… 2026

.....

.....

Signature of investigator Signature of witness

Permission to have all anonymous data shared with journals:

Please carefully read the statements below (or have them read to you) and think about your choice. No matter what you decide, it will not affect whether you can be in the research study, or your routine health care

When this study is finished, we would like to publish results of the study in journals. Most journals require us to share your anonymous data with them before they publish the results. Therefore, we would like to obtain your permission to have your anonymous data shared with journals.

Permission for sharing samples and/or information with other investigators:

Please carefully read the statements below (or have them read to you) and think about your choice. No matter what you decide, it will not affect whether you can be in the research study, or your routine health care.

In order to do the research, we have discussed, we must collect and store heart ultrasound and health information from athletes like you. Once we have done the research that we are planning for this research project, we would like to store your ultrasound data and health information. We may collaborate with other investigators from all over the world who can ask to use these data in future research. To protect your privacy, we will replace your name with a unique study number. We will only use this code for your sample and information about you. We will do our best to keep the code private. It is however always possible that someone could find out about your name but this is very unlikely to happen. Therefore, we would like to ask for your permission to share your ultrasound data and health information with other investigators.

Tick the Option you choose for anonymous data sharing with journals:

I agree to have my anonymous data shared with journals during publication of results of this study

☐ Signature_____

OR

I do not agree to have my anonymous data shared with journals during publication of results of this study

☐ Signature_____

Tick the Option you choose for sharing samples and/or information with other investigators: I do not want my heart ultrasound data and/or health information to be shared with other investigators

☐ Signature_____

OR

My heart ultrasound data and/or health information may be shared with other investigators for further analysis and future research in a field related to the study of cardiovascular adaptations in endurance athletes

☐ Signature_____

Appendix 4 **ATHLETE EXERCISE HISTORY QUESTIONAIRE**

2026 Munga MTB race (25-30 November 2026)

1.Name: ……..

ID number: …….

2.What is your age: ….. years

3.What is your sex: M F

4.Have you been diagnosed with any of the following conditions which would exclude you from the current study?

- heart palpitations or arrhythmias - Y/N
- disease of the 'electrical wiring of the heart' - Y/N
- ever had a pacemaker implanted - Y/N
- ever had heart surgery or had stents inserted - Y/N
- ever been diagnosed with "high blood pressure" - Y/N
- ever been diagnosed with 'heart muscle diseases' - Y/N
- ever been diagnosed with 'heart valve disease' - Y/N
- ever been diagnosed with 'heart vessel disease' ('coronary artery disease") - Y/N

5.Have you had any suspected viral illness including COVID-19 in the past 3 months?

6.Are you currently taking any medications?

If so please list:

……

……

……

…….

7.How many years have you been cycling: ……. years

8.How many Munga MTB races have you completed: ……..

9.How many of the following MTB races have you completed:

- ABSA Cape Epic ……
- Cape Pioneer Trek …...
- Freedom Challenge ……
- KAP sani2c ……
- Karoo Burn ……
- Wines2Whales ……
- Tankwa Trek ……

10.Which road or gravel cycling races have you completed incl number of finishes:

……

……

……

……

……

10.What was your average weekly training cycling volume in kms in the 12 weeks before the current 2026 Munga MTB race:

……

11.How long was your preparation for the current race: …… weeks

12.How many km did you cycle over the last 12 months: ……..… km

13.How many km have you cycled over your lifetime: ……..… km

14.For those who were until recently involved or are still involved in road/trail running:
For how many years have you been running: …… years

15.How many km did you run over the last 12 months: ……..… km

16.How many km did you run over your lifetime: ……..… km

17.How many marathons have you completed: …..….

18.How many ultramarathons have you completed: …..…..

19.what is your fastest time on a standard marathon: …… hours ……. minutes

20.What is your fastest 2Oceans Ultramarathon: …...… hours ……. minutes

21.What is your fastest Comrades Ultramarathon: …...… hours ……. minutes

22.Have your been actively involved in any other endurance sport such as canoeing, rowing, cross-country skiing, open water swimming, triathlon?

Please share details concerning exercise intensity, number of years, number of races:

……

……

……

……

Appendix 5.

Flow diagram of scheduled investigations during The 2026 Munga Heart Study

Day1.*Interview with the investigators (10-15 min) (on-line, telephonic, in-person)*

Day1.*Providing 'The 2026 Munga Heart Study' documentation (digital/in print), discussing and signing of study consent (5-10min) (on-line, telephonic, in-person)*

Day2.*Sport CRP, Netcare Blaauwberg, Sunningdale (10-15min)*

A.*Physical examination, obtaining laboratory samples (Pathcare, electrolytes, renal functions, hemoglobin/hematocrit, ironstudies)*

B.*Resting electrocardiogram (12-lead resting, 1st ultra-high frequency) (10min)*

C.*1st S10 InBody assessment (5-10min)*

D.*1st Resting (10min) + bicycle exercise echocardiography (20-25min)*

Day3.*Cape Radiology, Constantiaberg Medi-Clinic, Plumstead*

1st cardiac MRI study (30min)

Day3-4.*Sport CRP, Netcare Blaauwberg, Sunningdale (30min), after cardiac MRI cardiopulmonary bicycle exercise test/VO2max*

Race days 25-30thNovember The Munga

After finishing – in the race village, Wellington

A.2nd Ultra-high frequency ECG (5min)

B.2nd S10InBody assessment (5-10min)

C.2nd resting cardiac ultrasound (10min)

The 3 days after finishing The Munga.

Cape Radiology, Constantiaberg Medi-Clinic, Plumstead) (30min)

2nd cardiac MRI study