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Cardiac troponin concentrations following exercise and the association with cardiovascular disease and outcomes: rationale and design of the prospective TREAT cohort study

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ABSTRACT

Exercise can produce transient elevations of cardiac troponin (cTn) concentrations, which may resemble the cTn release profile of myocardial infarction. Consequently, clinical interpretation of postexercise cTn elevations (ie, values above the 99th percentile upper reference limit) remains challenging and may cause clinical confusion. Therefore, insight into the physiological versus pathological nature of postexercise cTn concentrations is warranted. We aim to (1) establish resting and postexercise reference values for recreational athletes engaged in walking, cycling or running exercise; (2) compare the prevalence of (sub)clinical coronary artery disease in athletes with high versus low postexercise cTn concentrations and (3) determine the association between postexercise cTn concentrations and the incidence of major adverse cardiovascular events (MACE) and mortality during long-term follow-up. For this purpose, the prospective TRoponin concentrations following Exercise and the Association with cardiovascular ouTcomes (TREAT) observational cohort study was designed to recruit 1500 recreational athletes aged ≥40 to <70 years who will participate in Dutch walking, cycling and running events. Baseline and postexercise high-sensitivity cTnT and cTnI concentrations will be determined. The prevalence and magnitude of coronary atherosclerosis on computed tomography (eg, coronary artery calcium score, plaque type, stenosis degree and CT-derived fractional flow reserve) will be compared between n=100 athletes with high postexercise cTn concentrations vs n=50 age-matched, sex-matched and sport type-matched athletes with low postexercise cTn concentrations. The incidence of MACE and mortality will be assessed in the entire cohort up to 20 years follow-up. The TREAT study will advance our understanding of the clinical significance of exercise-induced cTn elevations in middle-aged and older recreational athletes.

Trial registration number NCT06295081.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Exercise can result in cardiac troponin (cTn) elevations above the 99th percentile upper reference limit.
⇒ Exercise-induced cTn elevations can mimic the cTn kinetics of myocardial infarction, causing clinical confusion when evaluating athletes with elevated cTn concentrations following exercise.
⇒ Previous research suggested that exercise-induced cTn elevations following long-distance walking are associated with worse prognosis in older individuals.

WHAT THIS STUDY ADDS

⇒ Reference values for resting and postexercise cTn concentrations for male and female middle-aged and older recreational athletes engaged in walking, cycling and running.
⇒ The prevalence of (sub)clinical coronary artery disease in individuals with high versus low postexercise cTn concentrations.
⇒ Insight into the clinical utility of postexercise cTn concentrations to predict major adverse cardiovascular events and mortality during long-term follow-up.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The TRoponin concentrations following Exercise and the Association with cardiovascular ouTcomes study will advance our understanding of the clinical significance of exercise-induced cTn elevations in middle-aged and older recreational athletes participating in mass-participation walking, cycling and running events.

INTRODUCTION

Cardiac troponin (cTn) is a crucial biomarker in diagnosing acute coronary syndromes, along with clinical symptoms, ECG and/or imaging findings. Due to their cardiac-specific isoforms,[1] assessing cTn T or I subunits (cTnT and cTnI, respectively) is used to differentiate acute myocardial infarction (AMI) from

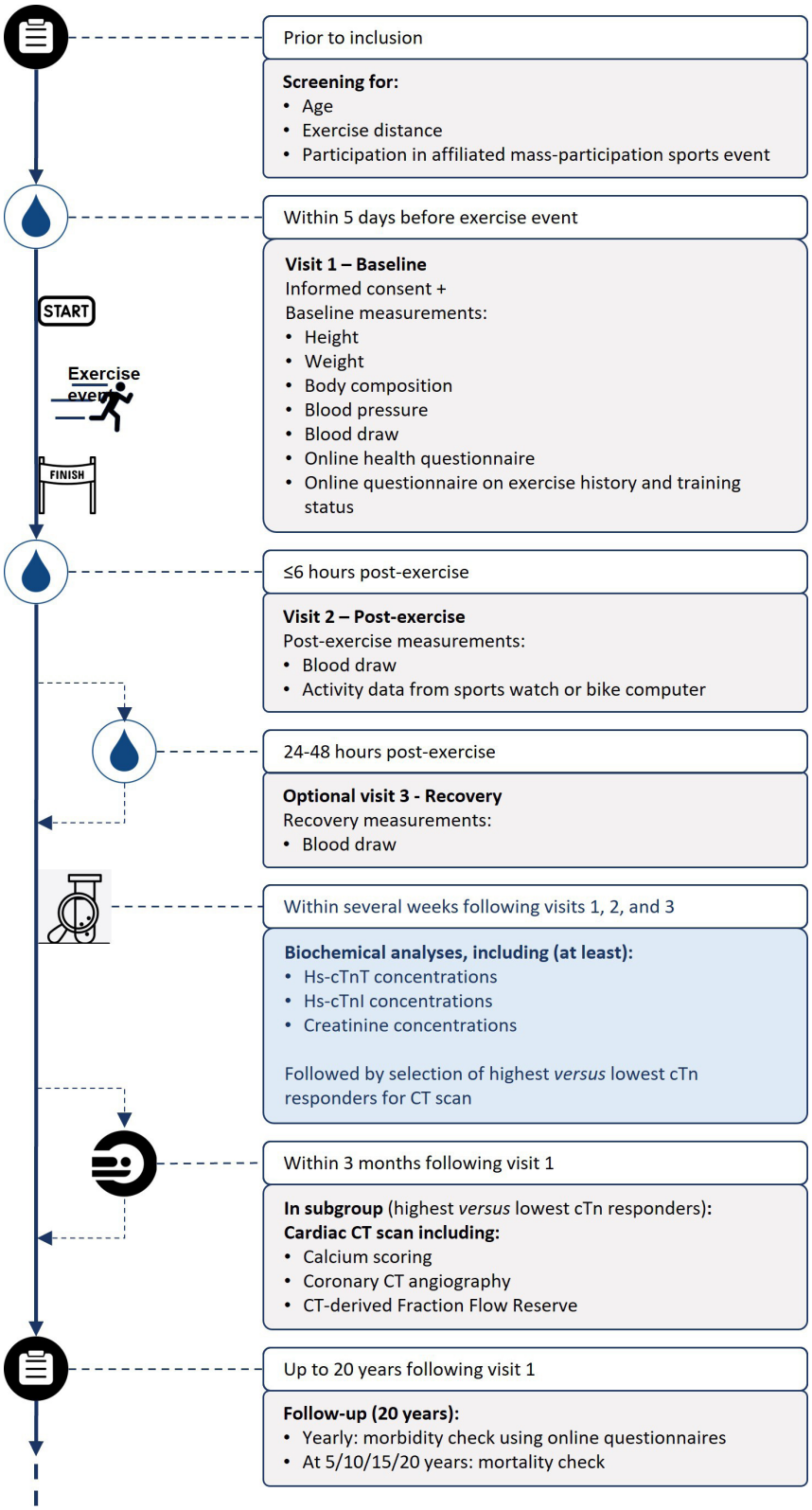

Figure 1 Overview of study design with procedures per study visit. Hs-cTnT, high-sensitivity cardiac troponin T; hs-cTnI: high-sensitivity cardiac troponin I.

other causes of chest pain in patients presenting at the emergency department (ED).[2] A single cTn value above the assay-specific 99th percentile (the upper reference limit, (URL)) indicates myocardial injury.[2] Furthermore, resting cTn concentrations are strongly associated with future morbidity and mortality in population and patient studies.[3 4]

Exercise also produces transient cTn elevations,[5] mimicking cTn elevations observed after AMI.[6] These exercise-induced cTn elevations are commonly reported

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
For phase 1: ► Participant in an affiliated mass-participation exercise event with a: – Walking distance ≥20 km – Cycling distance ≥100 km – Running distance ≥15 km ► Age: ≥40 and <70 years old ► Able to understand and perform study-related procedures	► (None)
For phase 2: ► Free from (known) cardiovascular diseases (defined as myocardial infarction, stroke, heart failure, peripheral vascular disease)	► Renal transplantation in the past ► Contrast nephropathy in the past ► eGFR<30 mL/min ► Atrial fibrillation ► Previous allergic reactions to iodine contrast ► Participation in other studies involving radiation ► Not willing to be informed about potential incidental findings from the CT scan

eGFR, estimated glomerular filtration rate.

in recreational athletes[7] and are unrelated to cardiac symptoms. For these reasons, exercise-induced cTn elevations were initially assumed to be benign. Still, outcome studies for exercise-induced cTn elevations were lacking, and mechanisms explaining exercise-induced cTn were largely unclear. Novel studies that could aid in discriminating between pathological (eg, acute coronary syndrome) and physiological exercise-induced elevations are, therefore, eagerly anticipated. Our first aim is to establish resting and postexercise reference values for recreational athletes engaged in walking, cycling or running exercise. For this purpose, we will collect data from a heterogeneous group (regarding age, sex and health status) of middle-aged amateur athletes participating in mass-participation exercise events.

Although exercise-induced cTn elevations are typically not associated with acute coronary symptoms, we hypothesise that postexercise cTn concentrations>URL may still represent myocardial injury due to underlying subclinical cardiac pathology. Indeed, participants with established cardiovascular disease (CVD) demonstrated a higher incidence of postexercise cTn elevations.[7] Furthermore, an increased risk for major adverse cardiovascular events (MACE) and mortality was found among long-distance walkers with postexercise cTn concentrations>URL compared with walkers with concentrations<URL.[8] Possibly, exercise could unmask cardiac vulnerability,[9] which might remain unnoticed under resting conditions. Indeed, cyclists with obstructive CAD (n=9) demonstrated higher cTn concentrations at 24 hours postexercise than healthy controls (n=109).[10] Thus, postexercise cTn elevations may indicate subclinical CAD. Therefore, our second aim is to compare CAD prevalence in a subgroup of participants free from known CVD (MI, stroke, heart failure, peripheral vascular disease) with high versus low postexercise cTn concentrations, matched for age, sex and sport type.

Only one study has assessed the clinical significance and predictive capacity of postexercise cTn concentrations for long-term cardiovascular outcomes.[8] Thus, large-scale follow-up studies targeting younger athletes and including different exercise types, such as long-distance running or cycling, are warranted. Accordingly, our third aim is to determine the association between postexercise cTn concentrations and the incidence of MACE and mortality during long-term follow-up.

METHODS

Study design

The 'cardiac TRoponin concentrations following Exercise and the Association with cardiovascular ouTcomes' (TREAT) study (NCT06295081) is a prospective cohort study among 1500 recreational athletes. The TREAT study consists of three phases (figure 1).

Phase 1: determining cTn reference values

Visit 1 (baseline) will be scheduled within 5 days pre-exercise, depending on the exercise event's organisation. Participants will be asked to refrain from vigorous exercise in the 48 hours before visit 1 to obtain valid baseline measurements. Height, weight, body composition and blood pressure will be measured using standard operating procedures, whereas a blood sample will be taken for biochemical analyses of cTn and creatinine concentrations. Participants will receive online questionnaires about their health status, lifelong exercise history and current training status. No interventions will occur during the mass-participation exercise event, but participants may register their exercise characteristics with a wearable or heart rate monitor. Visit 2 (postexercise) will occur within 6 hours after exercise cessation and consists of a blood withdrawal and registering exercise characteristics. Visit 3 (recovery) is optional and comprises collecting a blood sample 24–48 hours after exercise cessation.

Phase 2: cardiac CT scan

Following biochemical analyses, the highest versus the lowest cTn responders free from known CVD and with an estimated glomerular filtration rate (eGFR) ≥30 mL/min/1.73 m^2 will be invited for an extra visit consisting of a cardiac CT scan to assess the prevalence of (subclinical) CAD. The CT scan will take place within 3 months after inclusion.

Table 2 Summary of exercise event characteristics

Event	Sport type	Distance(s) (km)	Elevation (m)	Maximum number of athletes per event (n)	Weblink to event organisation
Seven Hills Hike	Walking	21/28	574/650	2000	www.zevenheuvelentrail.nl
Four Days Marches	Walking	34/40/52	60/60/70	47 000	www.4daagse.nl
Tour of Nijmegen	Cycling	120/165	461/525	3200	www.rondevannijmegen.nl
KlimClassic	Cycling	119/191	1100/2200	3500	www.klimclassic.nl
Seven Hills Trail	Running	21/28/42	574/650/967	3000	www.zevenheuvelentrail.nl
Stevensloop	Running	21.1	282	3000	www.stevensloop.nl
Seven Hills Run	Running	15	140	25 000	www.zevenheuvelenloop.nl

Phase 3: incidence of adverse health outcomes

All participants will be invited to complete an annual online health questionnaire to assess the incidence of MACE. Incident cardiovascular and all-cause mortality will be evaluated via data linkage to the Dutch population registry up to 20 years of follow-up.

Study setting and recruitment

We aim to recruit 1500 amateur athletes at Dutch mass-participation exercise events stratified by sport type (ie, walking, cycling, running). As exercise duration and exercise intensity are known predictors for the magnitude of exercise-induced elevations in cTn concentrations,[11 12] we will focus on long-distance events (ie, walking ≥20 km, cycling ≥100 km, running ≥15 km).

Recruitment will occur via official websites and newsletters of the associated exercise events and social media channels of the events and Radboud University Medical Center. Inclusion and exclusion criteria are summarised in table 1. For phase 1, male and female amateur athletes aged between ≥40 and <70 years participating in walking, cycling or running events will be eligible for inclusion. A list of the selected sports events and their characteristics is provided in table 2. For phase 2, additional inclusion and exclusion criteria apply because CT scans will only be conducted among the highest 6.6% vs lowest 3.3% cTn responders free from CVD (defined as MI, stroke, heart failure and peripheral vascular disease). The baseline and postexercise high-sensitivity cardiac troponin (hs-cTnT) and high-sensitivity cardiac troponin I (hs-cTnI) concentrations will be ranked to select participants. The concentrations receive a rank number from highest to lowest for the following biomarkers: hs-cTnT at baseline, hs-cTnI at baseline, hs-cTnT post-exercise and hs-cTnI post-exercise. The four rank numbers will be summed, and participants with the lowest sum score (and thus the highest cTn concentrations) will be considered high responders. Subsequently, we will select high cTn responders, and age-type-matched, sex-type-matched and sport-type-matched low cTn responders in a 2:1 ratio. For screening, we will assess participants' health questionnaires and eGFR. We will only select participants with an eGFR≥30 mL/min/1.73 m^2 for CT scanning because contrast nephropathy is unlikely to occur in these individuals. Before planning their CT scan, we will contact participants to confirm and schedule it.

Public involvement

We implemented a public involvement approach, allowing volunteers to sign up to assist the research team with data collection. Furthermore, we sought collaboration with exercise event organisers and media outlets to communicate the rationale and importance of our study and will continue doing so when disseminating our study outcomes.

Outcome measures

The primary outcomes for phase 1 are baseline and postexercise hs-cTnT and hs-cTnI concentrations. Other study parameters include hs-cTnT and hs-cTnI concentrations at 24–48 hours postexercise (recovery), participant characteristics, exercise characteristics (eg, exercise duration and intensity), cardiovascular health characteristics, lifestyle factors, physical activity and training characteristics, and other cardiac biomarkers (eg, creatinine, cholesterol, albumin, cardiac myosin binding protein C). A complete data catalogue is provided in table 3.

The primary outcome of phase 2 is the prevalence of (subclinical) CAD as determined using coronary artery calcium score, plaque characteristics (calcified/partially calcified/non-calcified) and coronary artery stenosis degree according to Coronary Artery Disease Reporting and Data System 2.0 (CAD-RADS 2.0).[13] Secondary outcomes include CT-derived Fractional Flow Reserve per coronary artery with ≥25% to 90% stenosis.

For phase 3, the primary outcome is the incidence of MACE and all-cause and cardiovascular mortality during the 20-year follow-up period.

Measurements

Anthropometrics

Height (in cm) will be measured with a wall-mounted measuring tape. Weight (kg) and body composition will be measured by the validated InBody 770 Body Composition Analyzer (InBody, Seoul, South Korea).[14] Participants will be asked to stand upright for at least 5 min and remove any items affecting measurements, such as shoes and jewellery. For safety reasons, those with a pacemaker

Table 3 Data catalogue table of the TREAT study

Category	Subcategory	Variables
Participant characteristics	Personal info	Sex (male/female); name; email address; phone number; place of residence; date of birth (dd-mm-yyyy); mass-participation sports event; distance (km). If included in the study: Participant identification number (TREAT…).
	Baseline measurements	Blood pressure: Systolic and diastolic blood pressure (mm Hg) at rest; heart rate at rest (bpm). Anthropometry: Height (cm); weight (kg); BMI (kg/m^2); body fat percentage (%); fat-free mass (kg); skeletal muscle mass (kg); fat mass (kg).
	Exercise characteristics	Exercise type (walking/cycling/running); exercise distance (km); start and finish time (hh:mm); pause time (hh:mm); exercise duration (hh:mm); average speed (km/hour); average speed (min/km); average heart rate (bpm). If used a sports watch or bike computer: .FIT-file or .CSV-file of activity.
	Medical history	General: level of education (primary education/preparatory vocational education/secondary vocational education/university of applied sciences/university bachelor/university master/PhD/other); ethnicity (Caucasian(European)/Afro-American/Asian/mixed/other); smoking (yes/no but I used to smoke/never); alcohol consumption (units/week); frequency of being active for≥30 min (days/week); ever used performance-enhancing substances (yes/no, if yes: which substances). Cardiovascular complaints and/or procedures: cardiovascular complaints(eg, dyspnoea, chest pain, fainting, palpitations)(yes/no); ever had coronary stenting or coronary artery bypass grafting (yes/no, if yes: reason of procedure, year and hospital where the procedure was performed). Diseases and/or conditions (if yes, date of diagnosis (dd-mm-yyyy): atherosclerosis (yes/no); stroke/TIA/CVA (yes/no); atrial fibrillation (yes/no); COPD/lung diseases (yes/no); embolism (anywhere other than lungs) (yes/no); erectile disorders (yes/no); intermittent claudication (yes/no); myocardial fibrosis (yes/no); fainting (yes/no); conduction disorders (yes/no); heart attack/myocardial infarction (yes/no); heart failure (yes/no); heart rhythm disorder (yes/no); heart murmur (yes/no); hypertrophic cardiomyopathy (yes/no); lung embolism (yes/no); kidney failure or kidney disorders (yes/no); myocarditis (yes/no); angina/chest pain (yes/no); rheumatic disorders (yes/no); diabetes mellitus (yes/no); thrombosis (yes/no); vascular dementia (yes/no); hypertension (yes/no); hypercholesterolaemia (yes/no); hypotension (yes/no); narrowed/occluded artery (yes/no); dilation of artery (aneurysm) (yes/no), disease of lymph nodes or vessels (yes/no), other diseases (yes/no, if yes: which disease and date of diagnosis (mm-yyyy). Family history of cardiovascular diseases in first-degree family members before age 60 years: sudden death<50 years (yes/no); myocardial infarction or cardiac arrest (yes/no); coronary stenting or coronary artery bypass grafting (yes/no); stroke (cerebral infarction or haemorrhage) (yes/no); high cholesterol (yes/no); dilation of an artery (aneurysm) (yes/no); narrowing of an artery (stenosis) (yes/no, if yes: which artery (carotid/coronary/legs/other). COVID-19: had coronavirus infection (yes/no/probably, if yes or probably: date of infection (dd-mm-yyyy) and how the infection was diagnosed (PCR test/CT scan/antigen test/self-test/general practitioner or medical specialist diagnosed/I only have a suspicion that I had a coronavirus infection). Medication use: participant uses medication regularly in the last year (yes/no, if yes: name of drug, dose (mg), frequency (doses/day), comments) Other: room for further comments regarding health in general
	Exercise history	Lifetime exercise history (if multiple sports: the following data will be collected per sport discipline): sport discipline; age started (years); age quit (years); frequency (days/week); frequency (months/year); average duration of a session (minutes). Participation in endurance races (how often participated in the following races/events): Zevenheuvelenloop (n); half marathon (n); marathon (n); ultra marathon (n); Nijmegen Four Days Marches (n); Kennedy march (80 km) (n); cycling events ≥120 km (n); Alpe d'HuZes (n); triathlon sprint (n); triathlon quart or half (n); triathlon whole/Ironman (n); other endurance races. Current endurance activities (if multiple sports: the following data will be collected per sport discipline): sport discipline; age started (years); age quit (years); frequency (days/week); frequency (months/year); average duration of a session (minutes). Strength: participation in strength training (yes/no). If yes: the frequency (days/week) of strength training, further specified as: Per type of muscle-strengthening exercise (using weight machines/bodyweight exercises/resistance exercises/holistic exercises): frequency (days/week); duration (0/<10/10–20/21–30/31–40/41–50/51–60/>60 min); muscle groups trained (legs/hips/back/abdomen/chest/shoulders/arms). Intensity at which the abovementioned muscle-strengthening exercises will be performed (0–10, 0 being extremely light, 10 being extremely heavy). Other: room for further comments regarding exercise history
Outcomes phase 1	Blood samples	hs-cTnT and hs-cTnI concentrations (ng/L) at baseline (≤5 days pre-exercise), postexercise (<6 hours after exercise cessation) and recovery (24–48 hours postexercise); creatinine (µmol/L); eGFR (mL/min/1.73 m^2); total cholesterol (mmol/L), HDL cholesterol (mmol/L), LDL cholesterol (mmol/L), albumin (g/L), cardiac myosin binding protein C (ng/L).

Continued

Table 3 Continued

Category	Subcategory	Variables
Outcomes phase 2	Coronary atherosclerosis burden	General: CT scan data (dd-mm-yyyy); average heart rate during scan (bpm); total radiation dose (dose length product (mGy/cm); beta-blockers administration (yes/no, if yes: dose (mg)); nitroglycerin administration (yes/no, if yes: dose (mg)); image quality (good/moderate/poor); FFR series obtained (yes/no, if yes: quality(good/acceptable/poor)); incidental findings. Coronary artery calcium score(=CACS), per coronary artery or other (LM/LAD/LCX/RCA/RI/Aorta/Valves/Other) and total: lesion count (n); Agatston score (AU); volume (mm^3); density (HU). Total CACS (AU); total volume (mm^3); total density (HU); MESA percentile (%). Coronary stenoses, per segment (1–18): maximum stenosis degree (0%/1–24%/25–49%/50–69%/70–99%/100%/non-diagnostic/vessel absent); the number of calcified plaques (n); the number of partially calcified plaques (n); the number of non-calcified plaques (n). Presence of myocardial bridging (yes/no); heart 'dominance' (PDA supplied by RCA/PDA supplied by LCX); general comments on CT scan (eg, complications). In general, not per segment: stenosis involvement score (n); stenosis severity score (n); plaque burden (P0/P1/P2/P3/P4); high-risk plaque features, per type(low attenuation plaque/positive remodelling/spotty calcification/napkin ring sign) (yes/no); modifiers, per modifier (non-diagnostic/having≥high-risk plaque features/ischemia/exceptions including coronary artery dissection, anomalous origin of the coronary arteries, coronary artery (pseudo) aneurysm, vasculitis, coronary artery fistula, extrinsic coronary artery compression, arterio-venous malformation, other causes)(yes/no); CAD-RADS 2.0 score without plaque burden and modifiers (0/1/2/3/4A/4B/5/N). CT-derived FFR per coronary artery (LM/LAD/LCX/RCA): FFR at the beginning of vessel (ratio); FFR at 1.5 mm diameter of vessel or most distal evaluable part (ratio); FFR at 20 mm distal from stenosis (ratio). FFR signs for ischaemia present (=FFR≤0.75)(yes/no); borderline ischaemia (=FFR 0.76–0.80) (yes/no); non-ischaemia(=FFR >0.80) (yes/no).
Outcomes phase 3	MACE	MACE (yes/no), if yes: date, type of event (myocardial infarction/stroke/heart failure/revascularisation (both acute and elective)/sudden cardiac arrest), hospitalisation (yes/no).
	Mortality	Mortality (yes/no), if yes: date of death (dd-mm-yyyy) and cause of death; cardiovascular death (yes/no).

Data are available for reuse on reasonable request via the corresponding author.
AU, Agatston units; BMI, body mass index; CAD-RADS 2.0, Coronary Artery Disease-Reporting and Data System 2.0; COPD, chronic obstructive pulmonary disease; CVA, cerebrovascular accident; eGFR, estimated glomerular filtration rate; FFR, fractional flow reserve; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; HU, Hounsfield units; LAD, left anterior descending; LCX, left circumflex; LM, left main; MACE, major adverse cardiovascular events; MESA, Multi-Ethnic Study of Atherosclerosis; PDA, posterior descending artery; RCA, right coronary artery; RI, ramus intermedius; TIA, transient ischemic attack; TREAT, TRoponin concentrations following Exercise and the Association with cardiovascular ouTcomes.

or implantable cardioverter-defibrillator will be excluded from the body composition measurement.

Blood pressure

Following ≥5 min of seated rest, at least three consecutive blood pressure measurements will be performed using an automatic device (Omron M3, OMRON Healthcare, Kyoto, Japan).

Blood sampling

Blood samples will be drawn from an antecubital vein. For visit 1, blood will be collected in serum tubes (serum separator tubes, SSTII advance, BD Vacutainer, Becton Dickinson, Franklin Lakes, New Jersey, USA) and lithium-heparin plasma tubes (plasma separator tubes, PSTII, BD Vacutainer). For visits 2 and 3, blood will be collected in serum tubes and BD Barricor lithium-heparin plasma tubes (Barricor plasma blood collection tube, BD Vacutainer), providing high-quality plasma suitable for gel filtration chromatography, a method to separate different cTnT fragments on molecular size.[15] All blood samples will be centrifuged according to the manufacturer's instructions, and aliquots will be stored at −80°C in the study location freezer. After biochemical analysis, blood samples will be stored for a maximum of 20 years in case additional analyses are warranted.

Questionnaires

Participants will receive an email with a personal weblink to an online questionnaire (Castor Electronic Data Capture, Castor, Amsterdam, the Netherlands). The questionnaire will collect general and cardiovascular health characteristics, cardiovascular risk factors, family history of CVD, COVID-19 status and medication use.

Furthermore, lifetime exercise history will be assessed, including sport type, years started and quitted, number of days per week and months per year, session duration, and intensity at which exercise was performed. To quantify lifetime exercise history, a validated questionnaire will be used.[16 17] In addition, the validated Muscle-Strengthening Exercise Questionnaire, which was long form,[18] was translated into Dutch and will be used to quantify participation in muscle-strengthening exercises. Furthermore, lifetime participation in endurance races will be asked.

Once exercise history data have been collected, a metabolic equivalent of task (MET) score will be assigned for all reported sports to calculate the lifelong exercise volume per sport by multiplying the MET score for the specific sport with the exercise volume (session duration×frequency/week), months of exercise per year and total years of training.

Exercise characteristics and data from wearables

During visit 2, participants will be asked the following exercise characteristics: exercise type (walking/cycling/running), distance covered (km), start and finish time, pause time if applicable, exercise duration if measured, average speed (km/hour or min/km) and average heart rate (bpm). In addition, we will derive activity data (such as continuous heart rate, speed and power measurements during exercise) from participants' (own) personal sports watches or bike computers. We will export .FIT- or .CSV files from participants' wearables at the study site or ask participants to send the raw data files to the study team via email.

cTn analyses

hs-cTnT concentrations will be analysed on a Cobas pure e402 (Elecsys Troponin T hs Gen 5 STAT, Roche Diagnostics, Mannheim, Germany). The limit of detection (LoD) is 3 ng/L, and the overall 99th percentile URL is 14 ng/L, according to the package insert. Hs-cTnI concentrations will be analysed on an ALINITY ci-series analyser (Alinity I STAT High Sensitive Troponin-I Reagent Kit, Abbott Diagnostics, Abbott Park, Illinois, USA) with an LoD of 0.7–1.6 ng/L and an overall 99th percentile URL of 26 ng/L, according to the package insert.

Other biomarker analyses

Creatinine concentrations will be analysed on a Cobas pure c303 (CREP2 Creatinine plus V.2, Roche Diagnostics) with an LoD of 5 µmol/L, LoQ of 10 µmol/L and expected values of 45–84 µmol/L for females and 59–104 µmol/L for males, according to the package insert. Creatinine concentrations in the baseline blood samples will be used to calculate the eGFR according to the CKD-EPI equation[19]. Furthermore, total concentrations of triglycerides, cholesterol, high-density lipoprotein and low-density lipoprotein (LDL) cholesterol, albumin and cardiac myosin binding protein C (cMyC) will be assessed. LDL cholesterol will be calculated according to the Martin-Hopkins equation.[20]

Cardiac CT scan

Volumetric CT scans will be performed on a 640-section CT scanner (Aquilion ONE PRISM, Canon Medical Systems, Tokyo, Japan). The participants will be asked to abstain from caffeine for 12 hours before the CT scan. Before scanning, participants will receive an intravenous cannula in an antecubital vein. Subsequently, participants will be positioned in the scanner and connected to an ECG monitor. Ideally, the heart rate is ≤60 bpm during scanning to avoid motion artefacts. Participants with a heart rate >60 bpm will be administered intravenous metoprolol (up to 10 mg). In addition, all participants will receive nitroglycerin sublingually (0.8 mg) to dilate their coronary arteries directly before CCTA scanning.

The imaging protocol consists of a scout view, followed by a prospective ECG-triggered coronary artery calcium (CAC) scan to detect and quantify the calcification in the coronary arteries. Subsequently, an ECG-triggered coronary CT angiography (CCTA) will be acquired after injecting iodinated contrast fluid with a concentration of 300, 350 or 400 mg/mL. The CCTA is performed with a widened data acquisition phase during diastole (70%–99% of the R-R interval) in patients with a heart rate ≤60 bpm. This widened window allows us to perform

Table 4 Cardiac CT acquisition protocol scan parameters

Participant preparation			
► No caffeinated products in the 24 hours before the scan. ► Intravenous access: 18G IV cannula, preferably in right arm ► Medication: – Intravenous metoprolol, up to 10 mg if HR>60 bpm – Sublingual nitroglycerin spray, 0.8 mg ► ECG sticker placement outside scan field of view ► Position: supine, feet first, arms above the head			
Contrast volume and injection rate			
Body weight	**Contrast fluid**	**Dose (mL)**	**Injection rate (mL/s)**
<75 kg	Iomeron 400	50	4
75–110 kg	Iomeron 400	60	4.5
>110 kg	Iomeron 400	70	5
Body weight	**Contrast fluid**	**Dose (mL)**	**Injection rate (mL/s)**
<75 kg	Iomeron 350/300	60	4.5
75–110 kg	Iomeron 350/300	70	5
>110 kg	Iomeron 350/300	80	5.5
Bolus tracking in descending aorta, halfway through the administered volume.			
Scanning parameters			
Parameter	**Coronary artery calcium score CT**	**Coronary CT angiography with FFR (if HR≤60 bpm)**	**Coronary CT angiography (if HR>60 bpm)**
Anatomical z-axis scan range (mm)	From carina (incl. coronary branches) to apex, 1 cm margin, 160	From carina to apex, 1 cm margin, 160	From carina to apex, 1 cm margin, 160
Scan mode, detector row width (mm)	Volume CT: 160×0.5	Volume CT, 160×0.5	Volume CT, 160×0.5
Localiser scans	Anteroposterior, lateral	From calcium score	From calcium score
Tube voltage (kVp)	120	80–120 (auto-kV)	80–120 (auto-kV)
Automated tube current modulation reference target noise level	SD: 55	SD: 40	SD: 40
Rotation time (s)	0.275	0.275	0.275
Breathing exercise	Inspiration (75%)	Inspiration (75%)	Inspiration (75%)
Focal spot size, mm^2	0.9	0.9	0.9
Data acquisition window RR interval:	70%–80%	70%–99%	30%–80%
IV contrast	No	Yes	Yes
Reconstruction method and kernel	Filtered back projection, FC 12	AiCE, Cardiac	AiCE, Cardiac
Reconstruction slice thickness and increment (mm)	3	0.5/0.25	0.5/0.25
Image reconstruction matrix	512×512	512×512	512×512

AiCE, Advanced intelligent Clear-IQ Engine; bpm, beats per minute; FFR, fractional flow reserve; HR, heart rate; IV, intravenous.

CT fractional flow reserve (CT-FFR) analysis to assess the haemodynamic relevance of coronary stenoses ≥25% to 90%. In patients with a heart rate of >60 bpm despite metoprolol administration, the scanning protocol will be adapted to a more narrow window, as CT-FFR will not be feasible within acceptable radiation dose limits in patients with a higher heart rate. The scanning protocols are displayed in table 4.

An established analysis software (TeraRecon iNtuition, TeraRecon, Durham, USA) will determine the Agatston score[21] and density and volume scores.[22] We will also calculate the Multi-Ethnic Study of Atherosclerosis percentile[23] based on participant characteristics. The CCTA will be evaluated and interpreted following the CAD-RADS 2.0[13] and Society of Cardiovascular CT (SCCT) guidelines.[24] The diagram of the SCCT guideline will be used to indicate where the stenoses are located.[24] Plaque type (if present) will be categorised as calcified, partially calcified or non-calcified. Calcified lesions consist entirely of calcified plaque and have a density >130 Hounsfield units (HU). Partially calcified plaques are composed of both calcified and non-calcified areas. Non-calcified plaques have an internal attenuation of <30 HU and consist entirely of non-calcified areas. In addition, the stenosis involvement score (ie, the total number of coronary artery segments with an atherosclerotic plaque from 18 segments) will be determined.

We will perform CT-FFR analyses to determine the functional significance of coronary artery stenoses ≥25% to 90%. CT-FFR postprocessing analysis will be performed on a dedicated workstation (Vitrea, Vital Images, Canon Group, USA). First, we will semiautomatically segment the coronary artery tree following the above-mentioned SCCT guidelines. Second, we will determine the CT-FFR values at the beginning and end of vessels and before and after a significant stenosis. A CT-FFR value ≤0.75 will be considered suggestive of ischaemia, between 0.76 and 0.80 indeterminate and a lesion-specific CT-FFR>0.80 suggestive of no ischaemia.[25] The most distal CT-FFR values will be computed in coronary artery segments with a diameter ≥1.5 mm.

An experienced cardiothoracic radiologist will assess all CAD-RADS classifications and stenoses' severity (or absence). Two researchers will independently perform all other CT analyses (Agatston score and CT-FFR). In case of discrepancies, the additional assessment by the cardiothoracic radiologist will be decisive.

Follow-up

The incidence of MACE will be assessed yearly, and cardiovascular and all-cause mortality will be assessed up to 20 years of follow-up. MACE will be collected from annual questionnaires and is defined as MI, stroke, heart failure, cardiac revascularisation (both acute and elective) or sudden cardiac arrest during follow-up. Medication use will be evaluated to ensure it matches the reported diagnoses. Mortality data will be retrieved from the Dutch National Register of Deceased Persons (National Death Registry).

Data management and accessibility

All data will be stored in a digital research environment (myDRE, anDREa BV, Nijmegen, the Netherlands) for at least 20 years, adhering to the General Data Protection Regulation, Good Clinical Practice and Good Laboratory Practice guidelines. Standard software packages will be used for data handling and statistical analyses (eg, Castor EDC, IBM SPSS, R, Microsoft Excel). In line with open science and the FAIR principles (Findable, Accessible, Interoperable and Reusable), data from the TREAT study will be made available for reuse on reasonable request via the corresponding author. A data catalogue is presented in table 3.

Sample size

As we aim to establish reference values for resting and postexercise cTn concentrations in participants of mass-participation exercise events, sufficient observations are needed to determine the 99th percentile URL in the whole cohort, as well as in specific subgroups (ie, sport type/sex/age). For this purpose, we aim to include 500 participants per sport type, a common sample size aligning with the Clinical and Laboratory Standards Institute recommendations.[26] Within each sport type, we strive to balance inclusion rates for sex (male/female) and age (40–49/50–59/60–69 years). If more people register for participation than we may include, we will distribute participants evenly over the different subgroups. Additional volunteers will be placed on a reserve list and can only participate if we have not reached the maximum number of inclusions for the particular sport type.

Statistical analysis

Statistical analyses will be performed by using SPSS statistics V.29 or higher (IBM). Collected data will be checked for normal distribution (visual and Shapiro-Wilk tests). Normally distributed continuous variables will be presented as mean±SDn and non-parametric distributed variables as median (IQR). Categorical variables will be expressed as numerical values and percentages. All statistical tests will be two sided, and p values <0.05 will be considered statistically significant.

Reference values for hs-cTnT and hs-cTnI will be established using descriptive statistics. Upper reference values will be defined at the 95th percentile and 99th percentile URL, including sex and sport-specific values following recommendations by Ichihara *et al*.[27] For this purpose, non-parametric analyses will be performed with outlier-adjusted 99th percentiles calculated using Tukey's outlier detection method. Furthermore, multiple regression analysis will identify factors that influence the test values in the reference population.

Conditional logistic regression analysis will compare the prevalence of CAD between the high versus low cTn responders. When comparing three or more groups (eg,

walkers vs cyclists vs runners), one-way analysis of variance (ANOVA) or Kruskal-Wallis tests will be used. Categorical variables will be compared using χ^2 or Fisher's exact tests. Because the high versus low cTn responders will be matched (if possible, at the individual level and otherwise at the group level), these paired data will be analysed using paired analytical methods. Conditional logistic regression will determine the prevalence of coronary atherosclerosis and lumen stenosis >50% (dichotomous variables) between participants with high versus low postexercise cTn concentrations. Coronary atherosclerosis will be compared between high versus low cTn responders by analysing plaque characteristics (calcified/partially calcified/non-calcified) using conditional multinomial logistic regression and CAC scores using ANOVA with a blocking factor, respectively. Following logarithmic transformation of CAC scores (ln(CAC+1)) and cTn concentrations (ln(cTn concentration) due to right skewness, univariate regression analyses and mixed model analyses will be used to study the association between postexercise cTn concentrations and CAC scores.

To evaluate and depict survival plots of incident MACE and mortality, Kaplan-Meier curves will be generated for cTn concentrations above and below a certain threshold (threshold to be determined). Logistic regression analyses will be used to study the relation between exercise-induced cTn elevations and the prevalence of MACE. Unadjusted and adjusted HRs for the incidence of MACE and mortality will be calculated using Cox proportional hazards regression analyses. Moreover, Kaplan-Meier curves and Cox proportional-hazards models will be generated to investigate the association between cTn concentration changes (ΔcTn=postexercise cTn-baseline cTn) and MACE and mortality, as was done previously by our research group.[8]

DISCUSSION

The TREAT study aims to improve the interpretation of exercise-induced cTn elevations and to assess its clinical importance. For this purpose, we will (1) establish resting and postexercise cTn reference values among recreational athletes engaged in walking, cycling or running exercise, (2) compare the prevalence of (sub)clinical CAD in athletes with high versus low postexercise cTn concentrations and (3) determine the association between postexercise cTn concentrations and the incidence of MACE and mortality during long-term follow-up. Given the large sample size and prospective study design, the TREAT study is expected to advance our knowledge of the clinical relevance of cTn elevations in amateur athletes.

Previous studies evaluating exercise-induced cTn release focused on young male athletes, had small sample sizes or were performed in controlled exercise laboratory settings.[7 15 28] These study characteristics limit the generalisability of findings as most recreational athletes participating in mass-participation exercise events are middle-aged, and more and more females join in vigorous endurance exercise. Therefore, we aim to include a large and diverse group of amateur athletes aged ≥40 to <70 years and of both sexes to study their cTn release following real-world exercise. Moreover, this will be the largest field study investigating exercise-induced cTn release in amateur athletes.

In current studies, there is significant heterogeneity in the prevalence of postexercise cTn values exceeding the 99th percentile URLs. An explanation could be that for each cTn assay, the 99th percentile URL is determined by the manufacturer using its own reference population. Furthermore, these assays were developed to aid in diagnosing acute coronary syndromes. Consequently, the current clinically used URLs do not help determine whether exercise-induced cTn elevations are (ab)normal. We take the initiative to establish reference values for pre-exercise and postexercise cTn concentrations to gain more insight into what might be expected in middle-aged amateur athletes.

The NEEDED study used CCTA to identify obstructive CAD and found that participants with occult obstructive CAD had prolonged cTnI elevation following strenuous exercise. However, the haemodynamic relevance of subclinical CAD is often unclear, especially in athletes, as coronary artery size and dilating capacity are increased among athletes compared with control subjects.[29] Tonino *et al* showed that only 35% of moderate stenoses (50%–70% lumen stenosis) are haemodynamically relevant (ie, FFR≤0.80) in patients with multivessel coronary artery disease,[30] which may be even lower in athletes. These findings highlight the need to assess the functional significance of stenoses found on CCTA. Therefore, we will extensively quantify coronary atherosclerosis and perform an additional CT-FFR on our participants with stenoses ≥25% to 90% on their CCTA.

cTn concentrations at rest predict cardiovascular morbidity and mortality in both population and patient studies. Exercise-induced cTn release has been considered the only exception and was believed to be benign because elevations are relatively mild and highly prevalent in apparently healthy athletes, usually returning to baseline within 24–48 hours[5], and there were virtually no studies investigating long-term outcomes. However, a recent study showed that exercise-induced cTnI elevations >99th percentile following 30–55 km of walking independently predicted higher mortality and cardiovascular events in older long-distance walkers.[8] The NEEDED trial did not confirm these findings as they found no higher risk for cardiovascular events in participants with postexercise cTn concentrations >99th percentile.[31] However, the low event rate and short follow-up duration (12 events in 1002 healthy subjects during 5-year follow-up) may have contributed to the fact that they found no higher risk in participants with cTn elevations. So far, no consensus has been reached on the clinical relevance of exercise-induced cTn elevations. With a follow-up of up to 20 years and a cohort of 1500 participants, the TREAT study will increase our knowledge of the potential value of cTn concentrations as a new marker for subclinical or future CVD.

Conclusion

The TREAT study will evaluate cTn release in male and female amateur athletes following long-distance walking, cycling and running events. We will establish reference values for pre-exercise and postexercise cTn concentrations in an amateur athlete population. Furthermore, we will compare the prevalence of (sub)clinical CAD in amateur athletes with high versus low postexercise cTn concentrations. During follow-up, we will investigate the association between cTn concentrations and cardiovascular events and mortality incidence.

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Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Ethics approval The Medical Research Ethical Committee region Arnhem-Nijmegen approved this study (NL79864.091.22), and the Declaration of Helsinki will be adhered to. Furthermore, Radboud University Medical Center's medical physicist approved the study protocol, and optimisation according to the ALARA concept (as low as reasonably achievable concerning radiation dose) was applied. Inclusion started in June 2022 and will continue up to June 2024.

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Data availability statement Data are available on reasonable request. In line with open science and the FAIR principles (Findable, Accessible, Interoperable and Reusable), data from the TREAT study will be made available for reuse on reasonable request via the corresponding author.

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