The clinical significance of exercise-induced cardiac biomarkers

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Scientific environment

This work is part of the North Sea Race Endurance Exercise Study (NEEDED) Research Group, led by Professor Stein Ørn, University of Stavanger and Stavanger University Hospital. Board members of this research group are Dr. Tor Melberg, Dr. Øyvind Skadberg and Dr. Rolf Bergseth.

Associated members of this research group include Professor Torbjørn Omland, University of Oslo, Professor Kenneth Dickstein, University of Bergen, and Associate Professor Kristin Moberg Aakre, University of Bergen. The project also cooperates with the University of Stavanger, represented by Professors Bjørn Auestad, Trygve Eftestøl and Kjersti Engan, and the University of Oslo, represented by Professor Thor Edvardsen.

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Contents

1. Acknowledgements

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2. Abbreviations and definitions

ACS	Acute Coronary Syndrome
AED	Automated External Defibrillator
AF	Atrial fibrillation
ATP	Adenosine triphosphate
BMI	Body mass index
BNP	B-type natriuretic peptide
CAC	Coronary artery calcium
CAD	Coronary artery Disease
CCTA	Coronary Computed Tomography Angiography
CI	Confidence interval
CK	Creatine kinase
CK-MB	Creatine kinase – myocardial band
CMR	Cardiac magnetic resonance imaging
CO_2	Carbon dioxide
CRP	C-reactive protein
cTn	Cardiac troponin
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
CV	Cardiovascular

ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
ESC	European Society of Cardiology
fsTnI	Fast skeletal muscle troponin I
Half-marathon	21 kilometre running event
HR	Hazard ratio
HDL	High-density lipoprotein
IPAQ	International Physical Activity Questionnaire
LAD	Left anterior descending artery
LDL	Low-density lipoprotein
LGE	Late gadolinium enhancement
Marathon	42 kilometre running event
MET	Metabolic equivalent of task
NA	Not available
NEEDED	The North Sea Race Endurance Exercise Study
NT-proBNP	N terminal pro Brain Natriuretic Peptide
REK	Regional Ethic Committee
SCA	Sudden Cardiac Arrest
SCD	Sudden Cardiac Death
SCORE	Systematic Coronary Evaluation

ssTnI	Slow skeletal muscle troponin I
WHO	World Health Organization

3. List of Publications

1. Skadberg Ø, Kleiven Ø**,** Bjørkavoll-Bergseth M, Melberg T, Bergseth R, Selvåg J,Auestad B, Greve OJ, Dickstein K, Aarsland T, Ørn S. Highly increased Troponin I levels following high-intensity endurance cycling may detect subclinical coronary artery disease in presumably healthy leisure sport cyclists: The North Sea Race Endurance Exercise Study (NEEDED) 2013. Eur J Prev Cardiol. 2017 May;24(8):885-894. Epub 2017 Feb 10.

2. Kleiven Ø, Omland T, Skadberg Ø, Melberg TH, Bjørkavoll-Bergseth MF, Auestad B, Bergseth R, Greve OJ, Aakre KM, Ørn S. Race duration and blood pressure are major predictors of exercise-induced cardiac troponin elevation. Int J Cardiol. 2019 May 15; 283:1-8. Epub 2019 Feb 23.

3. Kleiven Ø, Omland T, Skadberg Ø, Melberg TH, Bjørkavoll-Bergseth MF, Auestad B, Bergseth R, Greve OJ, Aakre KM, Ørn S. Prolonged cardiac troponin elevation following strenuous exercise may identify occult obstructive coronary artery disease. Eur J Prev Cardiol. 2019 Epub ahead of print.

4. Kleiven Ø, Bjørkavoll-Bergseth M, Melberg T, Skadberg Ø, Bergseth R, Selvåg J, Auestad B, Aukrust P, Aarsland T, Ørn S. High physical fitness is associated with reduction in basal- and exercise-induced inflammation. Scand J Med Sci Sports. 2018 Jan;28(1):172-179. Epub 2017 Apr 12.

4. Abstract

Background

Physical exercise induces changes in cardiac biomarkers associated with cardiac injury and inflammation. The ability of these changes to identify subjects at risk of sport-related cardiac events is largely unknown. This project aimed to identify the physiological pattern and predictors of the exercise-induced cardiac troponin (cTn) and C-reactive protein (CRP) response to exercise. A potential pathological response to exercise was explored in a subset of subjects.

Methods

The exercise-induced increase in cTn and CRP were studied in two cohorts of presumably healthy recreational athletes; The North Sea Race Endurance Exercise Study (NEEDED) 2013 (n=97) and 2014 (n=1002). Both studies obtained data before and within 24 hours after a 91-kilometre cycling competition ("The North Sea Race"). Coronary computed tomography angiography was used in a subset of subjects to identify a possible association between exercise-induced cTn elevation and underlying coronary artery disease.

Results

In Paper 1 (n=97), we determined the magnitude and time-dependent changes in cTnI following the North Sea Race. There was a continued increase in cTnI between immediately after the race and 3 hours following the race. This finding was important for the design of the main study. Also, three of the four subjects with highest exercise-induced cTnI values had significant coronary artery pathology.

In Paper 2 (n=1002), 84-92 % of subjects exceeded the 99th percentile of the cTn assay 3 hours post-race. At 24 hours post-race, 18-30 % still had cTn values above the 99th percentile. Lower race duration and higher systolic blood pressure were predictors of the exercise-induced cTn increase.

In Paper 3 (n=120), subjects were assessed by coronary computed tomography angiography (CCTA). Subjects with obstructive CAD (n=9) had significantly higher cTn levels at 24 hours after the race, but not at 3 hours after the race.

In paper 4 (n=97), the CRP response to exercise was determined. In this study, CRP continued to increase until 24 hours following the race. Physical fitness was found to significantly attenuate the exercise-induced CRP response. There was no association between exercise-induced levels of CRP and cTnI.

Conclusion

The present work identified the physiological pattern and predictors of exercise-induced cTn and CRP. More than 84 % of subjects had cTn levels above the 99th percentile cut-off. This finding supports the hypothesis that exercise-induced cTn increase is a physiological response in most subjects. Lower race duration and higher systolic blood pressure were consistent predictors of the exercise-induced cTn response. In a subset of subjects, a prolonged elevation of cTn was associated with occult obstructive CAD, suggesting a pathological pattern of cTn increase in these subjects. Lastly, physical fitness was inversely associated with the exercise-induced CRP response. No association between exercise-induced cTn- and CRP increase was identified.

5. Introduction

The World Health Organization (WHO) has identified physical inactivity to be the fourth leading risk factor of global mortality, only surpassed by high blood pressure, tobacco use and high blood glucose (1). Regular physical activity reduces all-cause and cardiovascular (CV) mortality by 20-39 % as compared to a sedentary life style (2). Increasing physical activity among the general population is therefore an attractive avenue for public health initiatives.

Adherence to regular physical activity is a considerable challenge. In a Norwegian study, only 32 % of adults fulfilled the current recommendations on weekly physical activity (3). Participation in recreational endurance exercise competitions such as marathons, ultra-marathons and cycling competitions serves as important motivational factors to uphold regular physical activity (4). The effect of vigorous exercise on the cardiovascular (CV) system, however, is not completely understood. In particular, sport-related cardiac events occur rarely, but regularly, and have potential catastrophic ramifications for those who are affected.

The European Sport Cardiology group recommends all athletes to undergo a pre-participation evaluation with a medical history and a resting 12-lead electrocardiogram (ECG) in order to minimize the risk of sport-related cardiac events (5). For athletes > 35 years of age, the main cause of sport-related cardiac events is coronary artery disease (CAD), and most victims of sport-related cardiac events are asymptomatic prior to the event (6-8). As such, these subjects are difficult to identify within the current evaluation algorithm. Research into novel strategies to identify subjects at-risk of sport-related cardiac events is therefore indicated.

Multiple studies have demonstrated significant increases in cardiac biomarkers following strenuous physical activity, with levels of cardiac markers increasing beyond the cut-off values of myocardial injury. The ability of these biomarkers to identify subjects who are potentially at risk of sport-related cardiac events is largely unknown. In this project, the physiological and potential pathological pattern of exercise-induced cardiac troponin (cTn) and C-reactive protein (CRP) are assessed.

5.1 Health benefits of regular physical exercise

There is strong evidence that regular physical exercise lowers all-cause mortality, coronary heart disease, high blood pressure, stroke, metabolic syndrome, type 2 diabetes mellitus, breast cancer, colon cancer, depression and falling (9). It also promotes higher level of cardiorespiratory and muscular fitness, enhanced bone health, better cognitive function and a healthier body mass and composition (9).

5.1.1 Physical inactivity

Physical inactivity is defined as a failure to meet the WHO recommendations on weekly physical activity (Figure 1) (9,10). In a global perspective, 31.1 % of adults are physically inactive (11).

WHO recommendation on physical activity for adults:

Minimum 150 minutes of moderate physical activity, or 75 minutes of vigorous physical activity per week

Muscle-strengthening activities on two or more days a week

Figure 1: WHO recommendations

In 2008, physical inactivity contributed to more than 5.3 million deaths worldwide (10). This corresponded to 9 % of premature mortality, and was a risk factor that contributed similarly to poor health as smoking and obesity. Inactive subjects are estimated to have a 16 % higher relative risk of incident coronary heart disease when compared to active subjects (10). The estimated relative risk increase for incident type 2 diabetes mellitus is 20 %, for breast cancer 33 %, colon cancer 38 % and all-cause mortality 28 % (10).

In a Norwegian cohort study conducted in 2015, only 1 of 3 adults fulfill the WHO recommendations for weekly physical activity (3). Social differences influenced the results markedly; male subjects who had a higher education were almost twice as likely to fulfill the recommendations as compared with male subjects with a primary school education only. In total, 62 % of time spent awake was inactive. The most common reason for inactivity was reported as “I do not have sufficient time to exercise” (37 %) (3).

5.1.2 Current recommendations for regular physical exercise

The WHO recommendations on weekly physical activity is outlined in Figure 1. Physical activity should be performed in bouts of at least 10 minutes duration, and includes activities during leisure-time, transportation and during working hours. Muscle strengthening exercises should include major muscle groups on 2 or more days a week.

There is a dose-response relationship with exercise and good health (9,12). As such, higher volumes of activity (up to 300 minutes of moderate exercise, or up to 150 minutes of vigorous exercise per week) is recommended for additional health benefits. Evidence of additional health benefits for exercise volumes greater than 300 minutes of moderate exercise per week is weak (9).

5.1.3 Challenges and motivation for regular physical activity

Physical inactivity is multifactorial, and depends on both individual factors such as age, sex and socio-economic status, as well as genetic, environmental and geopolitical factors (13). High crime rates and dense traffic may be obstacles to outdoor physical activities (13). Seasonal variations, as well as the absence of parks or recreational areas are also barriers to regular physical activities (3). Individual barriers also include limited time to exercise, lack of enjoyment of exercise, reduced self-efficacy, low social support, companionship, safety and/or a misconception of, or lack of knowledge of the physical activity guidelines (3,13).

Motives for continuing regular physical activity includes body-oriented motives, nature experiences, sociability, mental and physical well-being, stress-reduction and long-term positive health effects (14,15). Several studies also mention challenges and achievement as important motives for continued physical activity, particularly for men (4,14). In the “Running USA 2017 survey” (n=6 800), 62 % answered that training for a race was a major motivational factor to continue regular physical activity (16).

Between 2009-2014 a 13.3 % global increase in marathon participation has been reported (17). In line with this, several Norwegian endurance exercise competitions experienced considerable growth in this period (Figure 2).

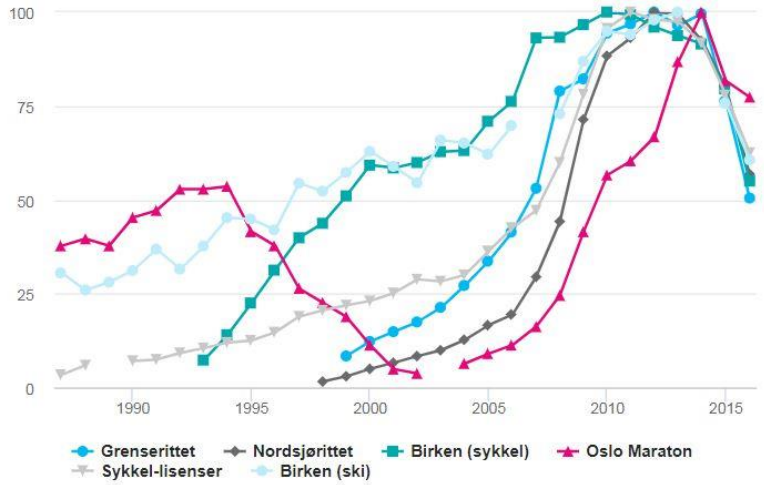


Figure 2: Participation in large Norwegian endurance exercise competitions between 1990-2015 (%). Grenserittet, Nordsjørittet (The North Sea Race) and Birken are cycling competitions, Oslo Marathon represents the largest marathon race in Norway and Birken (ski) is a cross-country skiing event. This figure was published by NRK.no 01.08.2017, and permission for reprint has been obtained (18).

5.2 Metabolic equivalents and exercise intensity

On a scale relative to an individual's personal capacity, moderate exercise is defined as physical activity of 5-6 on a scale to from 0-10, while vigorous exercise is defined as 7-8 on the same scale (9). This definition is commonly used in public health recommendations, as it is easy to understand and use. In absolute terms, however, exercise intensity may be defined as the ratio of the rate of energy expended during an activity to the rate of energy expended at rest. At rest, 1 metabolic equivalent of task (MET) is 3.5 ml oxygen/kg/min (9). Walking at a pace of 5.3 km/hour requires 3.3 METs. Running at a pace of 9.7 km/h requires 10 METs. By multiplying the METs required by the specific activity by the number of minutes that the activity is performed, provides MET minutes. Activities requiring 3-5.9 METs are classified as

moderate exercise, while activates requiring ≥ 6 METs are classified as vigorous exercise (9). 500-1000 MET minutes per week roughly corresponds to the recommendations on weekly physical activity by WHO.

Example: Walking at a pace of 5.3 km/hours for 1 hour would generate (3.3 MET x 60 minutes) 198 MET minutes. Running at a pace of 9.7 km/h for 20 minutes would generate (10 MET x 20 minutes) 200 MET minutes.

5.3 Basic exercise physiology

During rest, cardiac output is approximately 5 L/min, whereof 20 % is directed towards the musculature (6). During strenuous physical activity, the heart needs to increase its pumping capacity in order to supply oxygen and nutrients to peripheral organs. The heart is able to increase its pumping capacity (cardiac output) by 4-8 times during acute bouts of exercise (25-35 l/min), primarily by increase in heart rate, but also due to increased stroke volume and contractility (6,19,20).

Exercise starts by the brain initiating movement from the motor cortex (6). Concomitantly, the sympathetic nervous system is activated (6). The release of noradrenaline and adrenaline into the circulation leads to an increase in heart rate and widespread vasoconstriction, with the exception of blood flow to the exercising muscles and tissues without the ability for substantial vasoconstriction (cerebral and cardiac circulation). This increases systolic blood pressure. During exercise, the musculature needs about 84 % of the entire blood flow (6). In order to ensure adequate blood flow to the muscles, local factors within the muscle cause their blood vessels to dilate (6,19).

Exercise requires chemical energy to be converted into kinetic energy. This is achieved by splitting adenosine triphosphate (ATP). ATP is generated from metabolic substrates, such as glucose and fatty acids. During aerobic metabolism, one glucose molecule yields 36 molecules of ATP (6). This process consumes six oxygen molecules and produces six carbon dioxide (CO_2) molecules (6). The oxygen cost of

using fatty acids to produce ATP is higher, and the rate of energy production is much lower.

Ventilation is central in exercise physiology. Healthy subjects are capable of increasing ventilation from 6 L/min at rest to > 100 L/min during strenuous exercise (6). In addition, gas exchange is more efficient during exercise, due to increased pulmonary capillary perfusion and greater recruitment of alveoli (6). However, under anaerobic conditions, energy can still be produced, but the production of energy is far more costly. During anaerobic metabolism, one molecule of glucose will only produce 3 molecules of ATP (6). Anaerobic energy production also leads to acidosis, which needs to be buffered. This in turn leads to an increase in CO_2 production, and an increased respiratory gas exchange ratio (> 1.0), underscoring the lungs additional highly important function in buffering (6).

Exercise capacity is limited both by the ability to delivery of oxygen to peripheral tissues (central factors), and the ability to extract and use oxygen (peripheral factors) (6). In order to overcome these limitations, substantial cardiovascular, muscular and pulmonary adaptations may occur in athletes. As such, relatively untrained subjects will have smaller cardiac dimensions, less developed musculature and vasculature and a lower degree of exercise efficiency as compared with highly trained athletes. Different levels of exercise-induced adaptations might therefore yield different cardiac and peripheral responses to exercise. The association between physical fitness and exercise-induced increases in cardiac biomarkers, however, is largely unknown.

5.4 Long-term cardiovascular adaptations to strenuous physical activity

By engaging in regular physical exercise, adaptations of the heart occur to match the increased demands on the cardiovascular system. This may lead to a physiological increase in cardiac wall thickness and/or ventricular and atrial sizes; the so called “athlete’s heart” (20,21). Cardiac myocytes are terminally differentiated, and as such, the increase in cardiac mass is due to increased myocyte size, and not due to an

increase in the number of cardiac myocytes (19). Early cardiac adaptations, however, also includes adaptive changes within the cardiac myocytes, such as overexpression of isoform α of the myosin heavy chain and alterations of the Ca^{2+} regulatory system (6). Increased cardiac vessel size and number have also been described, contributing to increased coronary blood flow (6).

The increased left ventricular cavity in athletic subjects corresponds with an increased stroke volume. Left ventricular ejection fraction is usually unaffected by this physiological remodelling, but the mechanical systole might be shorter in athletes, as compared to controls, underscoring the increased cardiac efficiency in trained subjects (6,22). Similarly, right heart chambers might be enlarged, particularly among endurance athletes, but systolic function is unchanged (6). A small increase in aortic root dimensions might also be seen in athletic subjects (23,24).

The electrocardiographic changes associated with the athlete's heart are sinus bradycardia, first-degree atrioventricular block, incomplete right bundle branch block, early repolarization and isolated QRS voltage criteria for left ventricular hypertrophy (25).

There is ample evidence to support that exercise modality, intensity and duration are important factors in the development of exercise-induced cardiovascular adaptations (26). A landmark study by Morganroth et al. from 1975 used M-mode echocardiography to describe cardiac structure of athletes (27). They found that athletes engaged in endurance-type exercises had increased end-diastolic volume and mass (eccentric left ventricular remodelling) as compared to controls, while athletes in strength-type sports primarily had increased cardiac mass (concentric remodelling) (27). They hypothesized that endurance exercise had similarities with volume overload, as seen in aortic or mitral regurgitation, while resistance training was a pressure load condition, similar as with aortic stenosis (27). This finding has influenced how sport cardiologists have considered cardiac remodelling for decades. Newer data derived from contemporary echocardiography and cardiac magnetic resonance imaging, however, challenges the notion, with a finding of mostly normal

geometry, and with a mix of pressure and volume load both in endurance- and resistance-type exercise (26,28,29).

Cardiac remodelling in athletes is also highly influenced by sex, ethnicity and underlying genetic differences (6). Lastly, coronary and peripheral vascular adaptations are important as part of this exercise-induced cardiovascular remodelling complex, with increased vascular diameters, greater vascular compliance and increased capillary density in the trained musculature (6).

5.5 Risks associated with strenuous physical activity

Legend states that Pheidippides, a messenger sent to announce victory over the Persian army in the year 490 BC, ran from Marathon to Athens, shouted "we have won", and then died. It is said to be the origin story of the Marathon. This story is often told to demonstrate the risk involved with performing strenuous physical activity. It is, however, likely that this never actually happened, but that it stems from a poem by Robert Browning from 1879 (30). However, sudden cardiac death does occur occasionally during or shortly after vigorous exercise. These events frequently lead to significant media attention, as subjects are often young and healthy. Numerous studies have therefore addressed the incidence, cause and prevention strategies of sport-related adverse events over the past decades.

5.5.1 Short-term risks associated with strenuous physical activity

Injuries in conjunction with strenuous physical activity are common, particularly musculoskeletal and dermal/soft tissue injuries (31). For running, 6.8-59 injuries per 1000 hours of exercise have been reported, with medial tibia stress syndrome, Achilles tendinopathy and plantar fasciitis being the most common injuries (32). Mountain biking has been associated with 1.52 injuries per 1000 biking sessions, but hospital admission for these injuries were rare (5 % of injuries) (33). During half-marathons (21 km running), 5.15 per 1000 participants suffered a medical complication in a prospective 4-year study from South Africa (34). Only 0.51 per

1000 participant suffered a serious medical incident, and death occurred in 0.05 per 1000 participant (n=2) (34).

The incidence of sport-related sudden death has been reported to be 4.6 cases per 1.000.000 population per year for the general population of France (35). The incident of cardiac arrests during marathons or half-marathons in the United States of America has been reported to be 0.54 per 100.000 participants (36). In a retrospective analysis of Norwegian sport-related SCDs between 1990-1997, 23 cases were identified (37). 22 of these deaths occurred in males, and 11 were due to myocardial infarction (37).

Serious medical adverse events during strenuous exercise may, however, also be due to disturbances in fluid and electrolyte regulation, temperature regulation and serious injuries. In the South African study of half-marathon running, disturbances in fluid/electrolyte/acid-base and temperature regulation were more common than serious cardiovascular events (34). Following the Boston Marathon, an incidence of hyponatremia of 13 % was identified, however only 0.3 % had critical hyponatremia (s-sodium > 120 mmol/L) (38).

5.5.2 Long-term cardiovascular risks associated with strenuous physical activity

Although moderate exercise reduces the risk of a cardiovascular event from coronary artery disease, recent cross-sectional studies have suggested a dose-dependent relation between life-long exposure to physical activity and the burden of myocardial fibrosis (39-41), coronary artery calcification (39,42,43) and atrial fibrillation in highly active subjects (44-47). It is important to note that most of these studies are cross-sectional observational studies, and that a causal link between exercise and these pathophysiological processes has not been established.

Myocardial fibrosis

Several studies have described a high incident of focal myocardial fibrosis in veteran athletes. Breuckmann et al. (n=102 male marathon runners 50-72 years of age) found that 12 % of marathon runners had late gadolinium enhancement (LGE) on cardiac magnetic resonance imaging (CMR), as compared to 4 % of age-matched controls

(p=0.08) (41). Merghani et al. found that 14 % of their male athletes had LGE on CMR, compared to none of the controls (p=0.004) (39). Wilson et al. found LGE in 6 (50 %) of their veteran athletes (40). It is important to note that in studies comparing veteran athletes with a control group, the study subject characteristics of both groups are highly important when comparing results.

LGE in athletes has been predominantly described in in the interventricular septum and at the right ventricular inception point (non-ischemic distributions). For some of the athletes with LGE, however, occult CAD or silent myocarditis are suspected as the cause (39,48).

A study in rats performing high-intensity and long duration exercise for 16 weeks suggested a higher degree of fibrosis in the exercising rats, as compared to sedentary control rats, using histological and biochemical methods (49). These findings have not been reproduced in humans, but do suggest that exercise might induce adverse remodelling and increased risk of arrhythmias in some individuals.

Coronary artery disease

Veteran athletes have also been found to have a surprisingly high prevalence of coronary atherosclerosis (Table 1). Braber et al. (n=318) found that 52.5 % of their sportsmen had CAC > 0 Agatston Units (50). Möhlenkamp et al. found that 71.3 % of their marathon runners (n=108) had coronary artery calcium > 0 Agatston Units, with a median CAC of 36 Agatston Units (51). Both of these studies included male subjects only, and CV risk factors were prevalent. Aengevaeren et al. (n=284) also studied a male cohort of recreational athletes, and found CAC > 0 Agatston Units in 53 % of subjects and a median CAC of 36 Agatston units. A higher CAC score was identified in subjects who reported a higher exercise volume (43). Merghani et al. (n=152 athletes, 30 % female) found that 40 % of the total cohort had CAC > 0 Agatston Units in their athletic cohort (males: 48 %, females: 22 %), and the median CAC score in their cohort were 0 Agatston Units (39). Both Aengevaeren et al. and Merghani et al. noted that highly active subjects had a higher degree of calcified plaques, as compared to less active/control subjects, suggesting a more stable form of

CAD (39,43). Similar findings are reported in smaller studies (52,53), except for a small study of female subjects, where female marathon runners had lower CAC than sedentary female controls (54).

Table 1: Summary of previous studies on recreational/veteran athletes and coronary artery disease. NA = not available.

	n	Females	Mean age	Control group	CAC > 0 Agatston Units†	Median /mean CAC†	Obstructive CAD (athletes)
Möhlenkamp et al. 2008	108	0 %	57.2±5.7	Yes	71.3 %	Median: 36 (0-217)	NA
Schwartz et al. 2014	50	0 %	59.4±6.7	Yes, with more CV risk	NA	NA	NA
Tsifikas et al. 2015	50	0 %	52.7±5.9	No	48.0 %	Mean: 44±121	2.0 %
Braber et al. 2016	318	0 %	54.7±6.3	No	52.5 %	NA	5.3 %
Merghani et al. 2017	152	30 %	54.4±8.5	Yes	40.0 %	Median: 0	5.3 %
Aengevaeren et al. 2017	284	0 %	55.0±6.5	Compared different activity levels	68.0 %	Median: 9.4 (0-61)	NA
Roberts et al. 2017	26	100 %	56±10	Yes, with more CV risk	19.2 %	NA	NA
DeFina et al. 2019	21.758	0 %	51.7±8.4	Compared different activity levels	NA	Mean: 232‡	NA

† *CAC > 0 Agatston Units and median/mean CAC scores are given for the athletic cohort only. Some studies report median, others mean values, and these might therefore not be directly comparable. In the studies by Aengevaeren et al. and DeFina et al., data is given for subjects with the highest activity level.*

‡ *Estimated value based on the published data.*

The reasons for increased CAC in middle-aged recreational athletes are unclear. Shear stress forces and a hyperdynamic coronary circulation causing non-laminar flow during high-intensity exercise have been proposed to increase coronary artery calcification (39,43). Exercise-induced hypertension, systemic inflammation with repeated bouts of exercise and exercise-induced parathyroid hormone increases may also be involved in development of calcified CAD (39). Importantly, even with higher coronary artery calcium levels in highly active subjects than in the more sedentary population, active subjects did not have increased all-cause or CVD mortality as compared to sedentary subjects in a large 10-year follow-up study (42).

Atrial arrhythmias

Although moderate exercise is beneficial in preventing atrial fibrillation (AF), several studies have suggested an increased risk of AF in the most active individuals, particularly for men (44,55). The trigger is usually atrial ectopy (55). Atrial remodelling, with increased left atrial volume in athletes, has been associated with this increased prevalence of AF in athletes (56,57). Arterial hypertension, the most common cardiovascular risk factor among recreational athletes, has also been associated with increased risk of atrial arrhythmias (55). Other potential important modifiers of the arrhythmic risk in highly trained individuals might be the heightened vagal tone during rest, which leads to a shorter refractory period, and might enable macro re-entry tachycardia (55). An increased sympathetic tone during high-intensity exercise sessions might also modulate the risk of AF (55).

Atrial fibrosis has emerged as a hallmark of atrial remodelling, and has been associated with atrial fibrillation recurrence post-ablation (58,59). The association between exercise, increased left atrial volume and atrial fibrosis has not yet been fully elucidated.

Intermittent and underlying conditions

Myocarditis may cause sudden death, and athletes with symptoms suggestive of myocarditis should be evaluated with CMR (60). The presence of LGE on CMR has

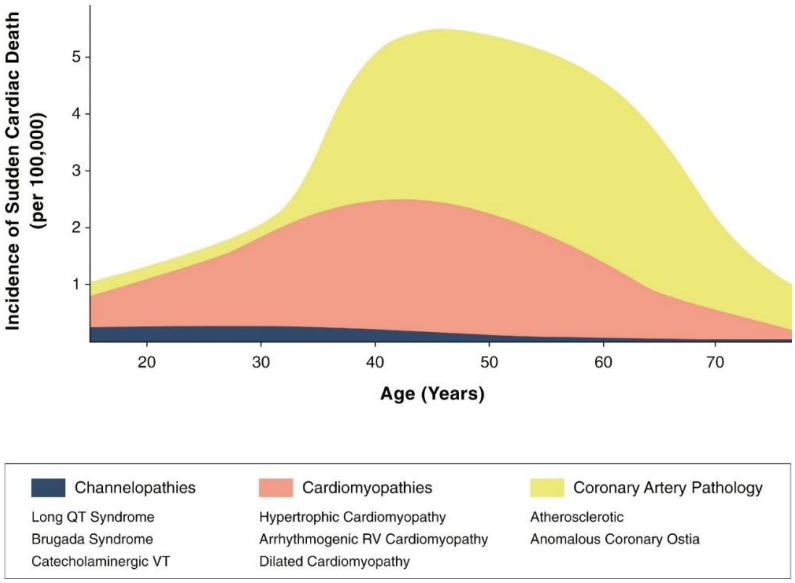
been found to be an independent predictor of mortality and major adverse cardiac events (61,62).

Hypertrophic cardiomyopathy, arrhythmogenic (right ventricular) cardiomyopathy, dilated cardiomyopathy, non-compaction cardiomyopathy, congenital abnormalities, channelopathies, pre-excitation, valvular disease and myocardial bridging are examples of underlying conditions that often increase the risk of sport-related SCD. Some of these conditions have overlapping phenotypes with findings associated with physiological remodelling, and there are different risks associated with the different conditions. The European Society of Cardiology (ESC) sport cardiology section has outlined recommendations on sport participation for these different entities (63,64).

5.6 Prevention of sport-related cardiac events

Cardiovascular adverse events during strenuous exercise relates to a number of different etiologies. For younger subjects and competitive athletes, channelopathies, congenital abnormalities and cardiomyopathies are common underlying causes of sport-related cardiac arrest. For middle-aged and older subjects (≥35 years of age), coronary artery disease is the most prevalent cause (Figure 3) (5,65). This is important to keep in mind when discussing strategies for preventing sport-related cardiac events.

Figure 3: Age-dependent changes in incidents and etiology of sport-related sudden cardiac death (65). The Figure is used with the permission of the publisher.



5.6.1 Young and competitive athletes

For athletes between 12-35 years of age, it is estimated that about 0.5-2 out of 100 000 athletes die suddenly each year (5,6). Men are more susceptible than women, and the risk increases with age (5).

Current European recommendations for pre-participation evaluation of athletes include a medical history, physical examination and a 12-lead ECG (5). Inclusion of other imaging modalities do not add substantial diagnostic power (5). The evaluation has been found to have a sensitivity of 75 %, and up to 30 % of all evaluated subjects may need further testing (66). As such, mandatory pre-participation evaluation of young athletes is controversial (66).

In Italy, introduction of a pre-participation evaluation for athletes was found to decrease sport-related SCD in from 3.6 to 0.4 per 100 000 person years over a 20 year period (67). Interestingly, these findings have never been reproduced in other cohorts (5). Currently the incidence of sport-related SCD is similar in France, USA and Italy,

suggesting that the pre-participation evaluation may not have been the cause of the reduced incident of SCD in Italy (66).

The Union of European Football Association, among several other sport societies, has introduced a mandatory pre-participation evaluation of their athletes, including an echocardiogram. Malhotra et al., published data from 20 years of screening young English soccer players, including data from 11 168 athletes. They found that 0.38 % of the screened athletes had cardiac disorders that were associated with sudden cardiac death. During the follow-up period, 8 deaths due to cardiac disorders occurred, whereof 6 (75 %) had normal cardiac screening results (68). This is in line with a Norwegian study, which found that six professional soccer players (1 % of the total cohort), all of whom had a negative screening at baseline, suffered a serious cardiovascular incident over a 8 year follow-up period (69). These findings underscore the uncertainty of a pre-participation evaluation strategy.

5.6.2 Senior athletes (> 35 years of age)

For athletes > 35 years of age, the incidence of sport-related sudden cardiac death has been reported to be 1/15.000 – 1/50.000 (6).

Pre-participation evaluation has been recommended for all active subjects ≥ 35 years of age who participate or plan to commence in strenuous physical activities (≥ 6 METs) (70). In this group, CAD is the most common cause of sport-related SCD (5). The evaluation of these athletes is similar as for younger subjects, but identification of CV risk factors is given more attention.

As for the younger athletes, there is considerable debate about the value of both a resting ECG and the maximal exercise test in subjects with no symptoms. Thus, the newer recommendations from 2017 states that for senior athletes, information should be given about the nature of cardiac prodromal symptoms, and that exercise stress testing is indicated if symptoms are present (5). Exercise stress test might also be considered in senior athletes with a high CV risk (5).

5.6.3 National practice

A required systematic pre-participation evaluation of sports participants has not been implemented in Norway. For Olympic athletes, The Norwegian Olympic Sports Center (Olympiatoppen) offers a health evaluation, which includes an ECG. They offer a repeat evaluation when the athlete is 20, 25 and 30 years of age. Also, football players at the highest level are evaluated regularly, due to requirements from the Union of European Football Associations (71).

5.6.4 Other preventive measures

Sport-related sudden cardiac arrest (SCA) is still unavoidable. Bystander cardiopulmonary resuscitation and use of publicly available automated external defibrillators (AED) are strong predictors of survival of sport-related cardiac arrests (35). As such, it is important that coaches, staff at sport events and laypersons are skilled in cardiopulmonary resuscitation and the use of AEDs. Readily access to AEDs in exercise facilities and during sport events is beneficial in preventing sport-related sudden cardiac deaths (35,72-75).

5.6.5 Future perspectives

There is consensus among both the American Heart Association and the European Society of Cardiology that “pre-participation evaluation for young competitive athletes is justifiable and compelling on ethical, legal and medical grounds” (6). Even so, there is considerable debate within the sport cardiology community about when to screen, which methods to use and how often a pre-participation evaluation should be carried out.

A majority of subjects with sport-related SCA are asymptomatic prior to the event (7,8). This knowledge, combined with the low sensitivity and specificity of a resting ECG in identifying underlying CAD are major limitations of the current strategy of pre-participation evaluation, particularly in the senior athlete population where cardiomyopathies and channelopathies are less common causes of sport-related cardiac events. As such, there is a need to identify novel methods to assess asymptomatic recreational athletes. As this population engage in strenuous exercise

regularly, both for competitions and training, the use of these exercise sessions to evaluate physiological responses to exercise is an attractive avenue of research in this area. The use of cardiac biomarkers could potentially unmask a pathological response to exercise; however, the clinical consequences of exercise-induced cardiac biomarker increase need to be further elucidated.

5.7 Cardiac biomarkers following strenuous exercise

Several biomarkers change following strenuous exercise. The underlying mechanisms, the time-dependent dynamics and clinical significance of these biomarker changes are largely unknown. Changes in markers of cardiac injury (cardiac troponins), inflammation (C-reactive protein), cardiac strain (B-type natriuretic peptides), lipid changes, hormonal changes and electrolytes have all been described earlier. Most previous studies, however, have been limited by small cohorts or use of older biomarker assays. In addition, many studies only sample before and immediately after exercise, and all-male cohorts are prevalent.

This current PhD project chose to focus on markers of exercise-induced cardiac injury (cTn) and inflammation (CRP). Cardiac troponins in this setting represent the acute cardiac stress-response to exercise, while inflammation is strongly associated with long-term cardiac events. Can these biomarkers assist in differentiating a physiological from a pathological response to exercise, and thereby aid in identifying subjects at risk of sport-related cardiac events?

5.8 Cardiac troponins

Assessment of cTn levels now constitutes the cornerstone in the diagnosis of myocardial infarction (acute myocardial injury), however, chronic myocardial injury with increased levels of cTn are also evident in patients with end-stage renal disease, in patients with arrhythmias, stroke, infections and even after non-cardiac surgery (76-81). In all these settings, increased cTn is associated with poor prognosis.

No long-term follow-up studies on cTn elevation following exercise had been published when this project was initiated, however, in 2014, Möhlenkamp et al. reported no adverse outcomes related to the exercise-induced cTn increase obtained immediately after a marathon in 74 marathon-runners (6±1 years of follow-up) (82). A very recent study by Aengevaeren et al. also assessed long-term outcomes of exercise-induced cTnI increase (83). In this study, a highly significant association was identified between all-cause mortality and major adverse cardiovascular events, and cTnI increase above the upper reference limit following a prolonged walking exercise in 725 participants (83). These findings suggest that exercise-induced cTnI increase may not be a completely benign, physiological response to exercise.

5.8.1 Molecular basis

Cardiac troponin (cTn) is an intracellular protein complex that consists of three subunits; Troponin I, Troponin T and Troponin C. Both cardiac specific isoforms of troponin I and T, and muscle troponin isoforms exist.

Within the cardiomyocytes, the cTn protein complex is attached to tropomyosin, and is part of the thin filaments. Together with the thick filaments, these form the sarcomere, the contractible unit. When an action potential from the sinoatrial node reaches the cardiomyocytes, an influx of Ca^{2+} occurs, and calcium binds to the troponin C subunit. This in turn leads to a conformational change in the cTn protein complex, and myosin binding sites on the actin filaments on the thin filaments are exposed, ultimately leading to muscle contraction (84). In addition to cTn bound to tropomyosin, cTn molecules are also present in a loosely bound form in the cytosol (85).

Troponin I is the actomyosin ATPase inhibiting subunit of the troponin complex. The gene that codes for cTnI is located on chromosome 19 (q13.4) (86). There are two other TnI isoforms: slow skeletal muscle TnI (ssTnI, 1q31.3) and fast skeletal muscle TnI (fsTnI, 11p15.5), both of whom are smaller molecules encoded for by genes on chromosome 1 and 11, respectively. Cardiac troponin I consists of 210 amino acids, and has a molecular weight of 24 kDa (86). The cardiac isoform of TnI has

similarities to ssTnI and fsTnI, but differs primarily by having a unique N-terminal extension (residues 1-30 for cTnI) (86). Post-translational modifications may be a significant factor in regulating the function of cTnI, particularly the process of phosphorylation, but this is not fully understood (86).

The troponin T subunit binds to tropomyosin, and has likely evolved from a TnI-like ancestor protein, prior to the emergence of vertebrates (87). Similar as for TnI, there are three genes that encode for troponin T; the cardiac isoform (1q32), the slow skeletal muscle isoform (19q13.4) and the fast skeletal isoform (11p15.5). As for TnI, the main structural difference between these isoforms is in the N-terminal region. Cardiac TnT has a molecular weight of about 37 kDa (84,87). Alternative splicing has been described for cTnT, and has been associated with cardiomyopathies. Post-translational regulation by phosphorylation has been described in vitro, however, this requires further investigation (87).

5.8.2 Analytical considerations

Different cTnI assays uses monoclonal antibodies specific to different epitopes of cTnI, and as such, standardization of cTnI measurements has proved difficult (88). Circulating cTnI is usually bound to cTnC (> 95 %), and detection of cTnI is influenced by proteolytic degradation, phosphorylation, heparin, and type of assay (88).

The high-sensitivity cTnT assay by Roche Diagnostics (fifth generation) uses monoclonal antibodies that binds to the central region of cTnT (88).

Both the high-sensitivity cTnI (Abbott) and the cTnT (Roche) assays used in this thesis have a low degree of imprecision (coefficient of variation < 10 % at the 99th percentile) (88). For Paper 1 in this thesis, the local overall 99th percentile for the cTnI assay was used, as determined by internal validation data at the Department of Biochemistry, Stavanger University Hospital (30 ng/L). For Papers 2-3 the overall and the sex-specific 99th percentiles was obtained by data from a more comparable

population of middle-aged blood donors (cTnI: overall 26 ng/L, males 28 ng/L, females 22 ng/L cTnT: overall 14 ng/L, males 16 ng/L, females 10 ng/L) (89).

After cTn enters the blood stream, degradation begins (90,91). This process is not fully understood, but might include phosphorylation, ubiquitination, complex-formation and binding to specific anti-cTn immunoglobulins (92). For patients with myocardial infarction, cTnT has been found both in an intact form (37 kDa), and in a primary fragment (29 kDa) and in several smaller secondary fragments (15-20 kDa) (91). The primary fragment is cleaved by the N-terminal end of the cTnT, while the secondary fragments are further cleaved by the C-terminal end (91,93). For subjects with end stage renal disease, and in a small study of marathon runners, only secondary cTn fragments were identified (94,95). Targeting the C-terminal end of cTnT in future assays might be useful to differentiate between cTnT forms in patients with chronic cTn elevations, physiological cTn elevations (i.e following exercise) and cTn due to irreversible cardiomyocytes damage. The major limitation in this research, however, is the limit of detection for the used methods; the mass spectrometry assay has a limit of detection of 1000-8000 ng/L, gel filtration chromatography has a limit of detection of about 70 ng/L (92,95).

The secondary cTn degradation fragments (<20 kDa) might possibly pass over the glomerular membrane for clearance (96). After an acute myocardial infarction, however, extra-renal clearance dominates in studies on rats (97). Extra-renal clearance might be associated with scavenger receptor clearance; however, this topic has not been fully elucidated.

5.8.3 Increase in cardiac troponin due to necrosis of cardiac myocytes

When the coronary blood flow is obstructed, ischemic cell death occurs. The subendocardial myocardium, which receives the least blood flow, is most susceptible to ischemic injury (98). With restoration of blood flow, myocardium might be salvaged. Timing, however, is important, and animal models have shown that after 6 hours of ischaemia, essentially no salvage occurs with reperfusion (99).

Early histological studies described myofibrillar contraction bands in necrotic cardiomyocytes; a process mediated by an influx of Ca^{2+} into the cytoplasm, leading the sarcomeres to contract tonically and consume the remaining ATP (99). This limits the cardiomyocytes' ability to pump out excess Ca^{2+}, and the cross-bridges between actin and myosin remain in an attached state. This in turn, leads to formation of sarcolemmal blebs, and eventually to disruption of the cellular membrane (99). Also, swelling of the mitochondria and destruction of the microvasculature occur (99).

After a transmural myocardial infarction, cTnI and cTnT are released within minutes after the cellular damage, and remain elevated for days after the infarction. This is in contrast to other intracellular molecules, such as creatine kinase and lactate dehydrogenase, where normalization of plasma levels is seen more rapidly. This prolonged elevation of cTn is most likely due to the binding between cTn and thin filaments of the sarcomere, which slows the washout of these molecules (96).

In the setting of an acute myocardial infarction, slight differences in the cTnI and cTnT kinetic pattern has been identified, with a prolonged or "biphasic" release of cTnT (100).

5.8.4 Increase in cardiac troponin due to apoptosis

Apoptosis of cardiomyocytes is cell death due to cell body fragmentation and engulfment by resident cells (101). Apoptosis can be triggered both by an extrinsic and an intrinsic pathway, and the mechanisms are complex (102). Since no intracellular content is expected to be released with apoptosis, it has been suggested that apoptosis cannot be a source of cTn elevation (96,103). Destruction of apoptotic bodies with release of cTn could possibly be a source for cTn release, however, this has never been proven (96). More research is needed on cTn release in relation to apoptosis (96).

5.8.5 Increase in cardiac troponin due to cell wounds

When cardiomyocytes are stressed by contraction, ischaemia, stretched by external forces or by beta-adrenergic stimulation, extracellular macromolecules have been detected in the cytoplasm of cardiomyocytes (96,104,105). These macromolecules enter the cell through gaps in the plasma membrane, so called cell wounds (96). Cells have mechanisms to protect against cell destruction due to cell wounds, and small holes in the plasma membrane are usually repaired within seconds (106). Mutations in genes involved in cell wound repair have been identified in three muscular dystrophies: Limb-girdle muscular dystrophy type 2B, Miyoshi myopathy and distal anterior compartment myopathy (107). For mice with mutation in these genes, cardiomyopathy and stable cTn elevations have been found (108). Cell wounds have therefore been proposed as a mechanism of cTn release from the cytosolic pool from viable cardiomyocytes, however, there is still limited understanding of this phenomenon (96).

5.8.6 Increase in cardiac troponin due to cross-reactivity with skeletal muscle

Both the cTnI assay by Abbott, and the cTnT assay by Roche use antibodies that bind to the cardiac isoforms of cTn. For the cTnT assay by Roche, cross-reactivity with skeletal muscle has been observed in patients with myopathies (109,110). No cross-reactivity has been described for the cTnI assay (109,110). This phenomenon of cross-reactivity was not found in a cohort of subjects with rhabdomyolysis (111). The effect of exercise-induced muscle injury in relation to cTnT cross-reactivity is uncertain.

5.8.7 Cardiac troponin in the general population and as a risk marker

Healthy blood donors have a median cTnI (Abbott) of 2.5 (2.4-2.5) ng/L with a 99th percentile of 28.9 (23.1-41.1) (89). The corresponding median cTnT (Rohce) concentrations in the same cohort was 2.7 (2.6-2.8) ng/L and a 99th percentile of 15.9 (14.6-18.3) ng/L (89). Baseline cTn increase with age and male sex (89,112). Higher

cTn levels have also been associated with body mass index (BMI), blood pressure, NT-proBNP, CRP and history of diabetes mellitus (113). An inverse relationship between smoking and cTn has also been described (113,114). Having higher baseline cTn is associated with an increased risk of future CVD (112,113,115).

5.8.8 Increase in cardiac troponin due to exercise

Cardiac troponin levels have been described to increase with exercise in several studies, the largest of whom is the study by Fortescue et al. (116,117). The exact mechanisms of exercise-induced cTn increase are largely unknown, but likely relate to cell wounds, as described in chapter 5.7.6 (118). It has been described that macromolecular exchange over the plasma membrane occurs in Petri dishes, and that this macromolecular exchange increases with contraction or stretching of cardiomyocytes (118). As regeneration of cardiomyocytes is limited, cTn increase due to necrosis is highly unlikely to be the main underlying cause of exercise-induced cTn increase (118).

At the time of the NEEDED study design, there was uncertainty about the magnitude and expected kinetics of cTn following prolonged cycling. With the use of older cTn assays, many study participants did not have detectable cTn levels following cycling events, despite several sampling time-points (119-121). Based on the study by Sharhag et al. a continued increase to three hours post-exercise was noted for cTnI, and possibly for cTnT following a cycling event in 45 subjects (122). These findings of a continued increase were supported by findings of studies on other exercise modalities (123,124).

The predictors of exercise-induced cTn increase had been reviewed by Shave et al in 2007, and they concluded that race duration and body mass were associated with detectable cTn following exercise (125). This meta-analysis included 26 studies, but was limited by assessing cTn immediately following exercise and by the use of older cTn assays. Eleven of these studies included only male subjects. As such, the predictors of exercise-induced cTn increase, particularly when studied as a

continuous variable, were largely unknown. No studies had assessed the relationship between coronary artery disease and exercise-induced cTn. Also, no long-term follow-up studies on exercise-induced cTn increase and clinical events had been published prior to the design of the NEEDED study.

In 2014, Möhlenkamp et al. published the first long-term follow-up study in this research area, and found no association between clinical endpoints and exercise-induced cTnI obtained immediately after a marathon in 74 subjects (82). There was also no association between coronary artery calcium (CAC) and exercise-induced cTnI (82). Only 6 marathon finishers, however, suffered an event (82). With underpowered studies, there is a risk of type 2 errors, and as such, this finding needed to be confirmed in larger cohorts.

A recent study published by Aengevaeren et al. followed 725 subjects after a 30-55 km walking exercise for a median of 43 months (83). In this study, median age was 61.4 years, and CV risk factors and CVD were prevalent (36 % and 14 % respectively) (83). cTnI was obtained immediately after the exercise, and 9 % of subjects were found to have a cTnI above the upper reference limit (0.04 µg/L, older cTnI assay used) (83). The combined endpoint of all-cause mortality, myocardial infarction, stroke, heart failure, revascularization or sudden cardiac arrest occurred in 62 subjects (9% of the cohort). Subjects with cTnI above 0.04 µg/L following the walking exercise had significantly higher risk of suffering a primary endpoint during follow-up (hazard ratio (HR): 5.21 (95 % confidence interval (CI): 2.96-9.17), $p<0.001$), also when adjusting for age, sex and CV risk factors, CVD and baseline troponin values (HR 2.48 (95 % CI: 1.29-4.78), $p=0.01$) (83). This study challenges the concept that exercise-induced cTn increase is a completely benign, physiological response to exercise. The finding, however, is not readily transferable to younger, healthier subjects engaged in strenuous activity, where cTn increases above the upper reference limit is highly prevalent. The role of later cTn sampling was also not elucidated.

5.9 C-reactive protein

C-reactive protein (CRP) is an inflammatory marker that has been highly conserved throughout evolution. It was discovered by Tillett and Francis in 1930 and named due to its reactivity with phosphocholine residues of C-polysaccharide on Streptococcus Pneumoniae (126). CRP together with serum amyloid P-component (SAP), are part of the highly conserved Pentraxin protein family. No conditions with human CRP deficiency has been identified, suggesting that this protein is highly important for our survival (127).

5.9.1 Molecular basis

The gene that codes for CRP in humans has been located on chromosome 1, and synthesis of CRP occurs primarily in the liver (127). The CRP protein complex is made up of a single polypeptide chain of 206 amino acids, and consists of five subunits that are arranged in a flat disc (127). CRP gene transcription is regulated by cytokines, especially Interleukin-1β, Tumor necrosis factor-α and Interleukin-6. The molecular weight of CRP is approximately 120 kDa (127).

CRP can be found in three isoforms: a multimeric form (≥ 10 subunits), the native pentameric form and as a monomeric CRP (1 subunit) (127). The physiological roles of the multimeric and monomeric CRP forms have yet to be established with certainty (127).

5.9.2 Analytical considerations

CRP is a readily available biomarker, and has a half-life of approximately 19 hours (127). It can be measured both in fresh, stored or in frozen plasma, and is stable in plasma or whole blood for at least 3 days (128). With the use of contemporary high-sensitivity CRP assays, concentrations as low as 0.15 mg/L may be detected. With the current widespread use, it has become an inexpensive test, used to test patients with suspected infections, inflammatory disorders and to determine CV risk.

Degradation of CRP occurs in the liver, and follows a stable exponential trajectory even in subjects with active infections (129,130). As such, the rate of CRP synthesis is the primary determinant of CRP increases.

5.9.3 Increase in CRP due to acute inflammation and injury

CRP exists only in trace serum concentrations in humans, and act as an acute phase reactant. It has the ability to increase 1000-folds in response to infection, tissue injury and inflammation (131).

CRP binds phosphocholine (and a variety of other ligands) at one side of the disc-like structure (the B face or the recognition face), and activate the classical complement system (C1q) on the opposite side (127,132). Binding of ligands is dependent upon calcium. By activating the complement cascade, phagocytosis occurs. As such, CRP is highly important in defending against bacteria.

CRP also binds to phosphocholine on injured cells, and activates the complement cascade (133). This is beneficial for most tissues, and leads to removal of dead cells. For tissues with a low rate of regeneration, i.e. heart muscle, this binding might be harmful (133). In the setting of a myocardial infarction, there are injured cells with a potential for survival in the area surrounding the necrotic zone. In animal models, CRP has been found to increase the infarct size and aggravate left ventricular dysfunction due to this binding to injured myocytes (133). An association between CRP increase post-myocardial infarction and infarct size has also been demonstrated in humans (134).

5.9.4 Increase in CRP due to chronic inflammation

Atherosclerosis is a complex disease, and inflammation is a vital part of the disease process (132). At the early phases of atherosclerotic plaque formation, leukocytes (monocytes) are recruited by proinflammatory cytokines (135). As the plaques develops, monocytes are differentiated into macrophages, and these macrophages engulf lipoproteins, and becomes foam cells (132,135). These cells secrete chemokines and cytokines that contribute to more leukocyte accumulation, smooth muscle cell proliferation and remodelling (132). Inflammation is also important in

degradation of the fibrous cap of the plaque, leading to plaque vulnerability and possible rupture (132). A ruptured plaque with formation of a thrombi is usually the origin of an acute cardiovascular event.

From the 1990s, several observational studies found that slight increases in CRP were independently associated with increased risk of CV morbidity and mortality (136-138). Statin-trials in this era also found that lowering LDL was associated with lower CRP levels, further inferring to the complex association between lipids and inflammation in atherosclerotic disease (139-141). Lowering lipids by the use of proprotein convertase subtilisin-kexin type 9 (PCSK9) inhibitors, however, has not been associated with decrease in CRP levels (142,143).

In order to establish a causal link between higher basal inflammation and future CVD, a study that targeted proinflammatory cytokines upstream to CRP were indicated. The CANTOS trial chose to target IL-1β, a cytokine involved in expression of cell surface proteins that increase inflammatory cell adhesion, and which stimulates vascular smooth muscle cell proliferation and upregulates IL-6 (135,144,145). In this randomized, double-blinded placebo-controlled trial, subjects were included if they had a CRP level ≥ 2 mg/L, and were followed for a median of 3.7 years. Subjects who received Canakinumab, the IL-1β antagonist, experienced a decrease in CRP level, but had no difference on LDL levels. A 15 % relative reduction in major adverse cardiac events was observed for subjects taking Canakinumab at 150/300 mg doses, as compared to the placebo group (p=0.007) (144). Further analyses showed that the effect was most pronounced in subjects who achieved a CRP concentration < 2 mg/L after the first dose, a finding that persisted throughout the study period (25 % risk reduction for major adverse cardiac events in this group) (146). It is important to note, however, that CRP reflects upstream processes, and there is no evidence that CRP is the cause of disease (133).

5.9.5 CRP in the general population and as a risk marker

Healthy subjects have a median CRP concentration of 0.8 mg/L, with the 90th percentile at 3 mg/L and the 99th percentile at 10 mg/L (131,147). CRP levels tend to

increase with age, obesity and current smoking (131). Cohort studies have found that baseline CRP levels < 1 mg/L implies a low risk of future CV risk. Levels between 1-3 mg/L are considered intermediate risk, while levels > 3 mg/L are considered high risk of future CV events (131).

5.9.6 Increase in CRP due to exercise

Exercise-focused interventional trials have been shown to lower CRP-levels, particularly when accompanied by weight loss (148,149). Physical activity level has also been associated with lower CRP-levels in community-based studies (150,151). A bout of acute exercise, however, generates an increase in CRP levels. This is an acute phase reaction, mediated by an exercise-induced increase in a number of cytokines, and in particular IL-6 (152). IL-6 is has been described to increase 100-fold following strenuous exercise, and is the cytokine with the earliest peak concentration (153,154). As described in chapter 5.8.1, IL-6 stimulates CRP synthesis in the liver.

Weight et al. studied 90 marathon runners, and found that CRP increased from 1.1-22.7 mg/L, $p<0.01$ (155). Several smaller studies have confirmed this finding, and exercise duration, modality, and increase in creatine kinase have been associated with the magnitude of the CRP increase (152). Some studies, however, have not been able to demonstrate a significant CRP-response to exercise (156,157). Scharhag et al. found that 4 hours of cycling only yielded an increase in CRP from 0.5-1.8 mg/L from baseline to 24 hours following exercise in their cohort of 12 young athletes (156). Prior to the NEEDED study, the effect of cycling on exercise-induced CRP was uncertain, and many of the prior studies had only included highly trained male subjects (152). A meta-analysis from 2015 of the acute effects of exercise in untrained subjects was only able to identify two studies on CRP (n=12 and n=14), and the exercise-induced CRP increase was not significant in these cohorts (158). As such, both the kinetics, magnitude and predictors of the exercise-induced CRP increase was largely unknown at the time of the design of the NEEDED study. Also, no studies on clinical outcomes in relation to exercise-induced CRP increase had been published.

Repeated bouts of exercise with its accompanying exercise-induced inflammatory response has been suggested to be related to increased CAC and CAD among highly active recreational athletes (39,159). Inflammation, and particularly IL-6, has also been associated with SCD in the general population (160-162). The link between exercise-induced myocardial injury, CAD and inflammation is therefore a compelling avenue of research within the field of sport cardiology.

5.10 Imaging modalities for identification of coronary artery disease in recreational athletes

Imaging modalities for assessment of CAD includes functional tests of ischemia and anatomical visualisation of the coronary arteries. The most commonly used functional tests are exercise ECG, stress echocardiography, myocardial perfusion scintigraphy, stress CMR and hybrid techniques. The anatomical assessments include coronary computed tomography angiography (CCTA), CMR coronary angiography and invasive coronary angiography.

Braber et al. reviewed the literature surrounding imaging of CAD in recreational athletes, and found that CCTA with calcium scoring was the modality with the highest diagnostic value (163). Calcium scoring from CT imaging is possible without the use of contrast, and with a low dose of radiation (<1 mS) (164). Pixels > 130 Hounsfield units are defined as coronary calcium, and quantified as Agatston units (165). The association between coronary calcium and degree of luminal obstruction, however, is poor. Subjects with a zero calcium score may also have severe plaques of a non-calcified nature (165). CCTA is a non-invasive method of determining the presence and severity of CAD. The method has a sensitivity of 95-99 % and a specificity of 64-83 % in diagnosing the presence of coronary artery disease (165). Radiation exposure is between 1-10 mS, and requires intravenous contrast (6). The quality of CCTA is influenced by heart rate, body weight and severe calcifications and prior stents. In order to achieve a heart rate < 65, short acting beta-blocking agents are used when appropriate. A meta-analysis found that cardiac death or MI occurred in 0.04 % of subjects with no CAD on CCTA, as compared with 1.29 % in

subjects with non-obstructive CAD and 6.53 % of subjects with obstructive lesions (166).

Functional imaging modalities for assessment of CAD, such as exercise-stress echocardiography (sensitivity of 80-85 %, specificity 80-88 %), stress CMR (sensitivity 79-88 %, specificity 81-91 %) and myocardial perfusion scintigraphy (sensitivity 90-91 %, 75-84 %) are robust tools to detect significant CAD, however, they are costly and have low availability (6,163,165). Exercise ECG testing is widely available, however, this test has a limited sensitivity (45-50 %) for detecting CAD, particularly in a cohort of subjects with low (<15 %) pretest probability of CAD (165).

Echocardiography alone has limited value in identification of CAD, but is an important imaging modality for assessing structural cardiac abnormalities (163). CMR allows for excellent assessment of cardiac function and of LGE without exposure to radiation (163). CMR angiography for anatomical assessment of the coronary arteries is a novel tool with the advantage of no radiation exposure. This method however, still offers limited visualization of the coronary arteries, and is currently most used in research (163,165).

Invasive coronary angiography is not a first-line test in low-risk subjects due to the higher risk of complications as compared with non-invasive imaging modalities. It uses intravenous contrast and exposes the patient to radiation (2-7 mS) (6). A valuable attribute of the invasive procedure is the possibility of estimating the fractional flow reserve in order to determine if a stenosis is clinically significant, and allows for the use of advanced intra-coronary imaging to assess plaque features.

5.11 Summary

Regular exercise is highly beneficial. Strenuous exercise, however, does carry a small risk of sudden cardiac events. More than 90 % of sport-related SCA occurs among

recreational athletes, and for senior athletes (> 35 years of age) CAD is the most common underlying cause (6,35). The majority of victims of sport-related SCA are asymptomatic prior to the event (7,8).

Current guidelines recommend pre-participation evaluation of all athletes who participate in strenuous exercise (5). The ability of a clinical history and a resting ECG to detect underlying CAD, however, is limited (66). As such, there is a need to identify novel methods to assess asymptomatic recreational athletes. As this population engages in strenuous exercise regularly, the use of these exercise sessions to evaluate physiological responses is an attractive avenue of research in this area.

Cardiac troponins are markers of myocardial injury, and increased levels have been associated with increased risk of cardiovascular mortality and morbidity, recently also following exercise (83). This biomarker is therefore of major interest in detecting potentially pathological responses to exercise. Higher basal inflammation has also been identified as a risk factor for CVD, and has been associated with SCA (132,160). Furthermore, it has been suggested that there might be an association between exercise-induced inflammation and increased CAD in athletes (39,159).

In this project, the physiological and potential pathological pattern of exercise-induced cardiac troponin (cTn) and C-reactive protein (CRP) are assessed.

6. Aims

6.1 General aims of the thesis

In this work, we aimed to identify the physiological pattern and predictors of exercise-induced cardiac troponin and C-reactive protein within 24 hours following strenuous exercise. Secondly, we aimed to identify a potential pathological response to exercise in a subset of subjects, and associate this with underlying coronary artery disease.

6.2 Specific aims

6.2.1 Paper 1

First, identify the magnitude and time-dependent changes of the exercise-induced cTnI response following the North Sea race. Secondly, to assess subjects with highly exercise-induced cTnI values for underlying CAD. This study was used to determine the design of the NEEDED 2014 study (Papers 2-3).

6.2.2 Paper 2

To identify the most important predictors associated with the cTn response following strenuous exercise in a large cohort of healthy recreational athletes.

6.2.3 Paper 3

To compare the exercise-induced cTn profiles in presumably healthy recreational cyclists with and without coronary artery obstruction identified by CCTA in order to separate a physiological from a pathological cTn response.

6.2.4 Paper 4

First, to identify the magnitude and time-dependent changes of the exercise-induced C-reactive protein response following the North Sea Race. Secondly, to identify the most important predictors associated with the CRP response following strenuous exercise. Third, to identify a potential relationship between exercise-induced CRP and cTn.

7. Materials and Methods

Paper 1 and 4 in this thesis used material from the NEEDED 2013 pilot study, while paper 2-3 used material from the main study, NEEDED 2014. Both studies centers on the completion of the North Sea Race, a 91 km cycling competition. The pilot study included 97 subjects, while the main study included 1002 subjects. Similar protocols for data collection were used both years, but up-scaled 1:10 for the main study.

7.1 Study organization, approval and registration

Professor Stein Ørn, MD, PhD initiated the NEEDED study. The board includes Dr. Tor Harald Melberg, MD, PhD of the Cardiology Department, Dr. Øyvind Skadberg, MD, Department of Biochemistry, Dr. Rolf Bergseth, MD, Medical officer for the North Sea Race, Torbjørn Aarsland, Research Department., and Jone Selvaag, Department of Biochemistry. The protocol was written by Ørn and Melberg, and approved by the board. The study was approved by the regional ethics committee (2013/550/REK vest), and registered in clinicaltrials.gov (NCT02166216 https://clinicaltrials.gov/ct2/show/NCT02166216).

7.2 Study populations, inclusion and exclusion criteria

The choice of study population was based upon the following assumptions; 1) Most sport-related cardiac events occurs among recreational athletes, 2) Cycling events attracts a more diverse cohort of recreational athletes, as weight is carried by the bike, and down-hill segments allow for periods of rest. This would allow subjects with a lower degree of physical fitness to participate, as compared to long-distance running-events. This was important, as we wanted a large cohort of athletes, ranging from those with very limited training experience, to those who had elite competition experience.

The North Sea Race was an ideal endurance exercise event; they attracted a large cohort each year, had a professional organization, and the organizing committee was

genuinely interested in participating in this research, as they had first-hand experience with sport-related cardiac events.

Study participants were recruited via the Internet, via the official web page of the race organizers (www.nordsjorittet.no). All participants in the North Sea Race in 2013 and 2014 were invited. In 2013, the Internet based invitation to participate was closed after 169 cyclists had signed up. A total of 111 cyclists signed the informed consent, and 97 cyclists completed the race and the study assessments. In 2014, registration was closed after 1250 subjects had signed up. In total 1002 participants were included in the main study.

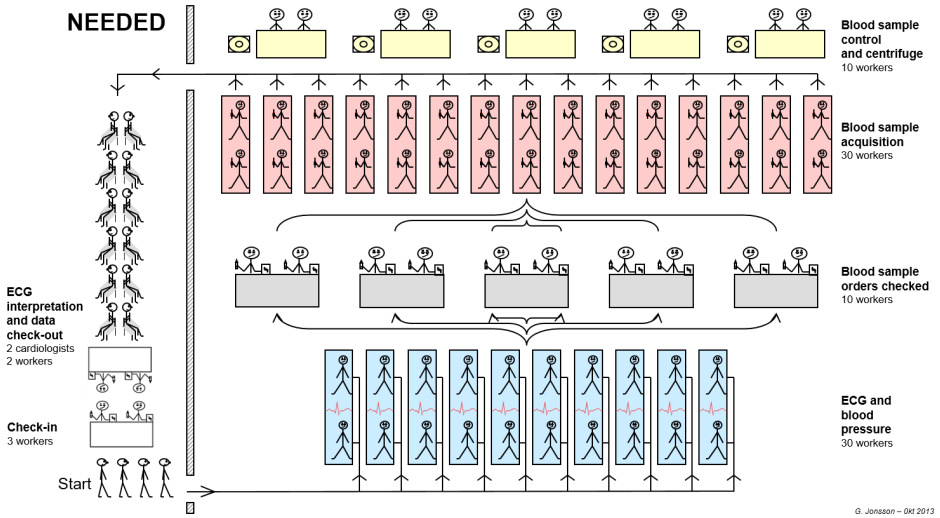
Subjects had to be ≥ 16 years of age, sign the informed consent and reside in Norway in order to be included in the studies. Subjects were excluded if they reported any CV symptoms or treatment for CVD (including CAD, stroke, diabetes mellitus or hypertension requiring medical treatment). Participants were also excluded if the baseline 12-lead ECG had signs of underlying CV disease: Q-waves (>3mm in depth or > 40 ms in duration in two or more leads except III, aVR and V1), T-inversions (>1 mm in depth in two or more leads in V2-6, II and aVF, or I and aVL), left bundle branch block or atrial or ventricular tachyarrhythmia. Subjects were excluded from the analyses if they did not complete the study assessments.

7.3 Data collection

Data collection in the 2014 study was based upon experiences from the 2013 study. Study subjects were first assessed 24 hours prior to the race (baseline). In 2013 subjects were then assessed both immediately and 3 hours after the race, while in 2014 assessments were obtained only 3 hours after the race. Both years, a final assessment was obtained 24 hours after the race.

Figure 5 outlines the logistical system that was developed for a comprehensive 30 minutes assessment of each of the study subjects in 2014. In 2013, a simplified version was used, with three lines.

Figure 5: Flow diagram of data acquisition before and after the North Sea Race in 2014. Logistics related to handling of study subjects at the finish line and transportation of samples to Stavanger University Hospital for analysis is not included in the diagram. A total of 157 study personnel were required to complete the study. The Figure was developed by G. Jonasson, and has been edited by Dr. Kleiven.



Assessments included registration, resting 12-lead ECG, resting blood pressure, body weight, blood sample acquisition and ECG interpretation and clinical assessment by a cardiologist. Waist circumference was obtained in 2014. Questionnaires were obtained at baseline, shortly after the race, 24 hours after the race and 1 week after the race, outlining training habits (including the standardized International Physical Activity Questionnaire (IPAQ-SF)), prior medical history, height, nutrition and post-race status. A fifth questionnaire was completed by female participants 2014, outlining hormone therapy, menarche, menopause, and childbirths. Race duration was registered electronically by the official organizer of the race. For subjects who used personal heart rate monitors, data on mean and maximal heart rate during the race was collected both years. In 2014, files from advanced heart rate monitors were collected electronically for further analysis when available (n=181).

The decision to not include an assessment immediately after the race in 2014 was based on logistical concerns: > 2000 assessment of approximately 30 minutes per

person over a 12-hour period was not considered feasible. As cardiac troponin levels were highest at 3 hours after the race, this was the chosen sampling time.

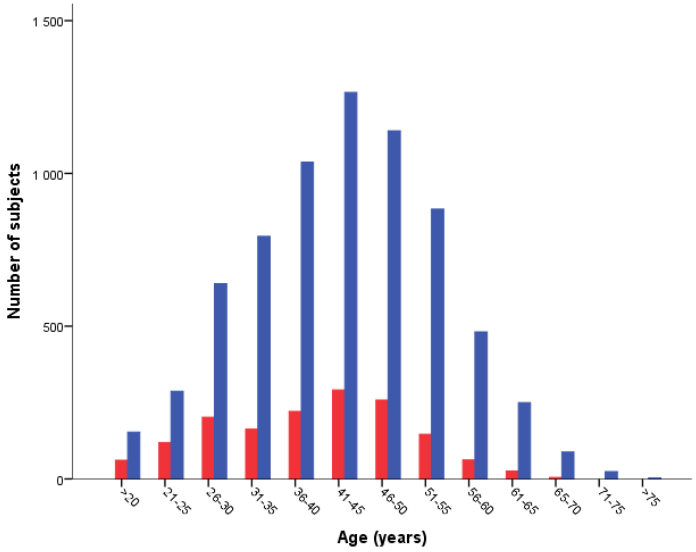
7.4 The North Sea Race

The North Sea Race is a cycling competition from Egersund to Sandnes (91km), and about 40 % of the track is on trails and mountain terrain. This race was first organized in 1998, and has grown in popularity, with a peak in participation in 2011 (Figure 2). The motto of this race is “For folk flest”, which loosely translates to “for ordinary people” and implies that limited prior exercise is needed in order to participate in this race. The organizers of the race also encourage local businesses to enroll teams and use the race as a team-building exercise. Race duration varies with weather conditions; however, a mean race duration between 4.0-4.5 hours is common.

During the race, safety is highly important. Six ambulances are placed at strategic points along the trail. Additionally, six teams with all-terrain vehicles (ATVs) are stationed in areas with difficult access. A total of 80 personnel covers the medical stations along the trail and at the finish line. The approximately 300 personnel posted along the trail are also trained in administering chest compressions and first aid, if needed.

Age- and sex distribution in the North Sea Race of 2014 is outlined in Figure 4. A total of 11 413 subjects signed up for the race in 2014, however, only 8678 (76 %) finished the race. The mean race duration was 4 hours and 13 minutes (men: 4:04±0:57, women: 4:51±1:03 hours).

Figure 4: Age and sex distribution among subjects who finished the North Sea Race in 2014 (n=8678, blue=men, red=women).



7.5 Blood samples

Venous blood samples were acquired from an antecubital vein at all assessments (4 times in 2013, 3 times in 2014). All samples were handles by professional medical personnel. Serum samples were centrifuged within 60 minutes at 2000 G for 10 minutes, and analyzed for creatinine, B-type natriuretic peptide (BNP), high-sensitive C-reactive protein (hs-CRP), low density lipoprotein, high density lipoprotein, creatine kinase (CK) and CK-myocardial band (CK-MB) on Architect c16000TM (Abott Diagnostics, Illinois, USA). For hematology, 3 ml K2 Ethylenediaminetetraacetic acid (EDTA) tubes were used, and the samples were analyzed on XE-5000 (Stsmex, Kobe, Japan). The tubes were stored at room temperature until analysis. Tubes without any additives were placed in room temperature and centrifuged after coagulation at 20000 G for 10 minutes. Following centrifugation, all blood samples were stored at +4 degrees C and transported to the Department of Biochemistry at Stavanger University Hospital for analysis.

Cardiac troponin I was analyzed using the high-sensitivity cTnI STAT assay by Abbott Diagnostics, analyzed on an Architect i2000SR (Abbott Diagnostics, Illinois, USA), with a lower limit of detection of 1.6 ng/L. Frozen samples were transported on dry ice to Haukeland University Hospital, Bergen, and cTnT was analyzed using a high-sensitivity cTnT assay on Cobas e601 (Roche Diagnostics, Switzerland) in serum that had not been previously thawed. The cTnT assay had a limit of blank of 3 ng/L.

CRP was measured in serum. The local analytical variation (CV) is <3 % at 24 mg/L.

In 2013, glycated hemoglobin (HbA1c) was measured at baseline on Variant Turbo II (BioRad Laboratories, California, USA). Copeptin (analyzed on Kryptor Compact Plus, B.R.A.H.M.S. GmbH, Thero Fisher Scientific, Hennigsdorf, Germany) was also obtained in 2013, but not in 2014.

7.6 Coronary computed tomography angiography

Coronary calcification and CCTA was obtained using a Siemens Somatom Definition Flash Dual Source. Slice acquisition parameter was 0.6 x 128 mm. Those with heart rate more than 60 beats per minute were given atenolol or metoprolol tartrate prior to examination. All were given 0.8 mg nitroglycerine sublingual before the scan. A scout scan was performed from under the tracheal bifurcation to the diaphragm, followed by CT calcium score scans with gantry rotation 280 ms, 12 kV, 80 mAs. A two-phase injection of Omnipaque 350 mg/ml at a rate of 6 ml/sec followed by 0.9 % saline with high pitch or prospectively ECG triggered protocols were administered. CCTA was reconstructed with slice thickness of 0.6 mm medium smooth tissue convolution and iterative reconstruction.

Obstructive CAD was defined as ≥ 50% luminal stenosis in the left coronary artery, the right coronary artery, the circumflex artery, or a diagonal or marginal branch. The CCTA examinations were independently assessed by two experienced radiologists, blinded to the cTn findings. Non-obstructive CAD was defined as a luminal

narrowing of 1-50%. Subjects with obstructive CAD on CCTA were referred for conventional coronary angiography and treated with percutaneous coronary intervention or coronary artery bypass grafting, as determined by the cardiologist performing the angiography.

In the pilot study (NEEDED 2013), study subjects were referred for a coronary computed tomography angiography (CCTA) or to a conventional coronary angiography, if they had cTnI > 500 ng/L at any time following the race (predetermined cut-off based upon our own non-published data from elite cyclists). We considered this cut-off to be the upper boundary of a physiological cTn response. As both subjects with cTnI > 500 ng/L had coronary artery disease, a post-hoc decision was made to scan subjects with descending cTn values until no more CAD was identified in 5 consecutive scans. Based on the findings from the pilot-study, a cut-off of 201 ng/L was determined as a guide to the planning of CCTA assessments in the main study.

In 2014, it was considered that including the 80 subjects with the highest cTnI values at any time following the race would roughly comply with the proposed cut-off of 201 ng/L from the pilot-study (8 subjects had cTnI > 201 ng/L in the pilot study). 40 subjects, matched for age and sex, with moderate cTnI increase after the race were assessed as a reference group. The reference group was an amendment to the protocol, and CCTA was obtained 3-12 months later in these subjects (see Chapter 10.12 for further details).

7.7 Statistical analysis

Descriptive statics was used in all the Papers, using mean±SD to describe normally distributed continuous variables, and median (25^{th}-75^{th} percentile) for variables with markedly skewed distributions. The Shapiro-Wilk test was used to test for normality. Number (%) was used to describe frequencies. Chi-Square test, Fisher's Exact test, Student t-test or Mann-Whitney U test was used for comparison of groups, as

appropriate. Wilcoxon test or Kruskal-Wallis test was used when comparing related samples. A two-tailed p-value of <0.05 was considered significant.

Bivariate correlations were assessed using Spearman's rank correlation due to the markedly skewed distributions. Multiple linear regression analyses were performed in Paper 2 and 4, using a backward variable elimination procedure. Receiver operating characteristics was used in Paper 3.

For statistical analyses, the statistical software programs SPSS versions 23 and 24, GraphPad Prism 7 and R were used.

7.8 Sample size and power analysis

We aimed to include about 100 subjects in the hypothesis generating pilot study (NEEDED 2013). This would be comparable to similar studies on marathon runners (167,168). The pilot study focused mainly on kinetic and magnitude of cardiac biomarkers. CCTA in this cohort was obtained in subjects with the highest cTnI following the race, and was not a primary aim of this study. An overall methodological aim of the pilot study was to assess how many subjects it would be feasible to assess the following year, for the main study.

The NEEDED 2014 study was designed to answer several research questions, of which the most important was considered to be the follow-up studies to assess long-term consequences of exercise-induced cardiac biomarker increase. As such, we wanted the largest possible cohort, as CV events among healthy middle-aged subjects are rare. Based on the experience from the pilot-study, we did not consider it feasible to assess more than 1000-1100 subjects, even when omitting one sampling time-point following the race. Due to the large number of subjects who dropped out during the pilot study recruitment, we chose to close the inclusion after 1250 subjects had registered for the study. In total, 1002 subjects were included. These subjects will be followed at 5, -10 and 20 years for a primary endpoint of all-cause death, myocardial infarction, revascularization, sudden cardiac arrest, stroke and heart failure admissions. Cardiac arrhythmias and cancer in this cohort will also be assessed.

As a subgroup analysis, the 80 subjects with the highest cTnI increase following the 2014 race were examined by CCTA (Paper 3). This was based upon findings from the pilot study. As an amendment to the protocol, 40 subjects with moderately elevated cTn were also examined by CCTA to determine to prevalence of CAD in this group. No similar studies had been published, and as such, there was insufficient data to perform adequate sample size and power calculations calculation in order to assess difference in prevalence of CAD in the High-cTnI vs the Reference group. Based on findings in the pilot study, three subjects in the High-cTnI group (cTnI > 201, 3/8) had significant coronary pathology; however, only two had obstructive CAD. If we considered the prevalence of CAD in the pilot study (25-37.5 % in the High-cTnI group), and assumed a prevalence of 5 % in the Reference group, a sample size of 40 subjects in the Reference group would yield a power of > 80 %.

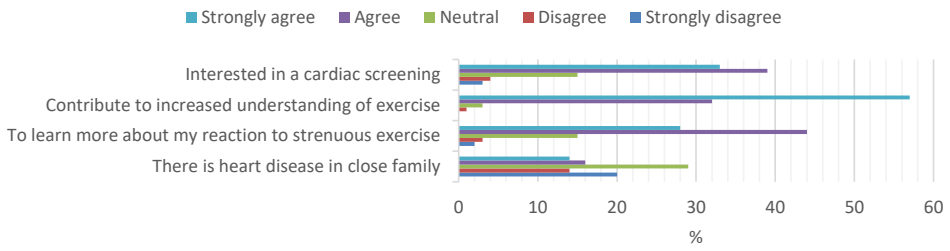
8. Ethical considerations

This study has been approved by the Regional Ethic Committee (2013/550/REK vest). Study subjects were recruited among the participants in the North Sea Race, and all participants signed informed consent prior to enrollment. No specific subgroup was recruited. All participants voluntarily subjected to blood sample acquisition three times (four times in the pilot study). Participants with atypical findings, either on the ECG or due to blood sample analysis, were referred for further testing. Subjects included into the CCTA sub-studies were subjected to some radiation. The risks of this study were considered reasonable weighed against potential benefits.

Data was collected and stored on secure servers of the Research Department of Stavanger University Hospital. Paper produced during data acquisition (ECGs, consent forms etc.) are stored in locked archives in the basement of "Forskningens Hus", Stavanger University Hospital. Blood samples are stored in the local biobank at Stavanger University Hospital, in accordance with current standards.

The athlete's perspective on this study was important to us. One week following the NEEDED 2014 study, a follow-up questionnaire was collected. Subjects were invited to give their feedback, and were also asked about their motivation to participate. A total of 88.3 % of the study participants agreed or strongly agreed that contribution to increased understanding of exercise physiology was an important motivational factor (Figure 6). Of note, 71.6 % of the participants agreed or strongly agreed that a thorough health screening was of importance to them. This is of cause of importance when interpreting the results of this study, in relation to possible selection bias.

Figure 6: Participants motivation for participating in the NEEDED 2014 study.



The main ethical consideration encountered during the work with this project related to findings of CAC and even CAD in otherwise healthy subjects.

Subjects with obstructive and non-obstructive CAD were advised to start statin therapy (IA recommendation for patients with stable CAD) (165). This decision warranted discussion for several reasons; first, statin therapy to subjects with established CAD is clearly beneficial on a community level, however, could this evidence be readily transferred to otherwise healthy, highly active recreational athletes, some with very small plaques and low CAC score? Second, could we potentially risk an increased incidence of adverse effects of statins, and in particular rhabdomyolysis, in our cohort, based on the high activity level? Some case reports suggest that statin-users might be vulnerable to exercise-induced rhabdomyolysis (169-171). Third, would this treatment of a subgroup of our subjects affect the results of the long-term follow-up studies, and as such obscure important outcomes? We concluded that it was in the best interest of the individual participants with coronary atherosclerosis to be prescribed a statin, and considerations of possible adverse events and study outcomes were secondary concerns.

For subjects without CVD, antiplatelet therapy is not indicated (III B recommendation) (164). However, there is a 1A recommendation for low-dose aspirin therapy for subjects with stable CAD in the 2013 guidelines on stable CAD (165). The WHO defines CVD as "… disease of the blood vessels supplying the heart muscle" (172). As such, a subject with a non-obstructive plaque probably falls within the definition of CVD. Stable CAD is defined in the 2013 ESC guidelines as

"episodes of reversible myocardial demand/supply mismatch, related to ischemia or hypoxia, which are usually inducible by exercise, emotion or other stress and reproducible – but, which may also be occurring spontaneously" (165). The guidelines then list four different underlying mechanisms of stable CAD: 1) Plaque-related obstruction of epicardial arteries, 2) focal of diffuse spasm of normal or plaque-diseased arteries, 3) microvascular dysfunction and 4) left ventricular dysfunction caused by prior acute myocardial necrosis and/or hibernation (165). As such, our subjects with non-obstructive coronary plaques and no symptoms of angina might not fulfill the definition of stable CAD. In the new 2019 guideline on chronic coronary syndromes, aspirin therapy for subjects without a history of MI or revascularization, but with definitive evidence of CAD on imaging may be considered (IIb, C) (173). No references support this statement. For subjects with CAC > 100 Agatston Units, numbers needed to treat to prevent one cardiac event has been reported to be 173 for subjects with a Framingham risk score <10 %, and 92 for subjects with a Framingham risk score > 10 % (174).

This 2013 recommendation of aspirin in subjects with stable CAD is based upon three studies; one is a randomized controlled trial published in 1992 and two are meta-analyses (published in 1994 and 2002) (165). In the randomized controlled trial, no tests of ischemia or angiography were required (175). Subjects below 30 years of age were not included. A 34 % relative reduction of MI and sudden death was noted among those who also received aspirin, p=0.003 (175).

We found that the published studies were not readily transferable to our cohort of asymptomatic recreational athletes with non-obstructive plaques, and as such we were skeptical of advising aspirin therapy to these participants. Recent studies have also challenged the use of aspirin in primary prevention in subjects with CV risk factors (176-178). Subjects with obstructive CAD were advised to start aspirin therapy.

The decision to refer subjects with obstructive CAD or highly increased coronary artery calcium score on CCTA to an invasive coronary angiography was made by the

project leader, in accordance with standard clinical practice at Stavanger University Hospital. The decision to revascularize some of these subjects was made by the invasive cardiologist responsible for the examination, in accordance with the patient. To date, however, the benefit of revascularization as an initial treatment approach of subjects with stable CAD, even for symptomatic subjects, is uncertain (179,180). Some of these subjects had very severe CAD, and wished to continue their level of physical activity, which probably influenced the decision to revascularize. Also, when asked in more detail, some did report atypical symptoms that could be interpreted as angina. Two subjects from the NEEDED 2014 study also had ECG changes following the race, which would support that ischemia was likely in these subjects (new T-wave inversions).

Conflicts of interest have been disclosed by some of the co-authors on the Papers, mainly due to co-operation with Abbott and Roche. Furthermore, Dr. Bergseth is the Principal Medical Officer of the North Sea Race. None of the subjects with conflicts of interest participated in the data analysis of these studies.

As researchers, we are ethically bound to share the results of our research to the study participants, fellow researchers and colleagues, as well as to the general population. The results of the NEEDED studies have been communicated to the study participants through the North Sea Race website, as well as in invited talks. The studies have also received coverage in both local, social and national media. The cardiology community has been well informed about the study, with presentations given at > 10 national and international congresses.

9. Results

Paper 1 outlines the cardiac troponin I (cTnI) response after the North Sea Race in the 97 subjects included in the pilot cohort. This paper also included data on CCTA on the 13 subjects with the highest cTnI response after the race. Paper 2 is an analysis of the predictors of the exercise-induced cTn response in the total NEEDED 2014 cohort. Paper 3 outlines findings on CCTA in the 80 subjects with the highest cTnI after the race in 2014, and compares this with CCTA data from 40 subjects with moderate cTn increase. Paper 4 outlines the CRP response in the pilot cohort, and identifies the most important predictors of this response.

9.1 Study populations

Baseline characteristics of the 2013 (Paper 1 and 4) and 2014 cohorts (Paper 2) are outlined in Table 2. Paper 3 uses data on a subgroup of subjects from the NEEDED 2014 cohort.

Table 2: Excerpt of baseline characteristics of subjects included in the pilot study (NEEDED 2013, Paper 1 and 4) and in the main study (NEEDED 2014, Paper 2).

	NEEDED 2013 (n=97)	**NEEDED 2014 (n=1002)**
Age, years	43±10	47 (40-53)
Sex, male	74 (76)	782 (78)
Body mass index, kg/m^2	25.3 (23.4-28.0)	25.3 (23.7-27.3)
Systolic blood pressure, mmHg	138 (129-152)	136 (126-148)
Diastolic blood pressure	77 (71-85)	79 (73-86)
Race duration, hours	4.2 (3.6-4.7)	3.7 (3.4-4.2)

Most of the included subjects were engaged in recreational sports only, and 25 % of the included subjects in 2014 exercised between 0-2 times per week (Figure 7). Study recruitment for Papers 2-3 is outlined in Figure 8. In total, 24 % of subjects ≥ 35 years of age in the NEEDED 2014 had at least one CV risk factors, with BMI ≥ 28 kg/m^2 being the most prevalent (Figure 9).

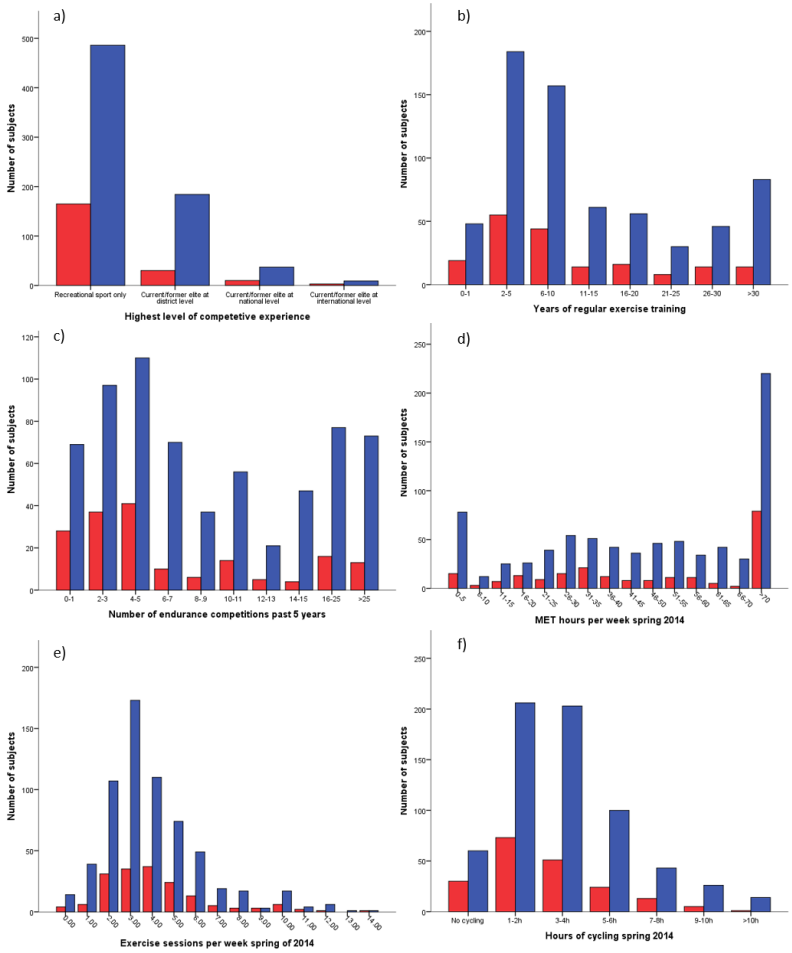


Figure 7: Exercise training experience in men (blue) and women (red) of the 2014 cohort: a) Highest level of competitive experience, b) Years of regular exercise training, c) Number of endurance exercise competitions past 5 years before the study, d) Self-reported MET hours per week, obtained by the International Physical Activity Questionnaire, e) Exercise sessions per week during spring of 2014, f) Hours of cycling-specific training during spring of 2014.

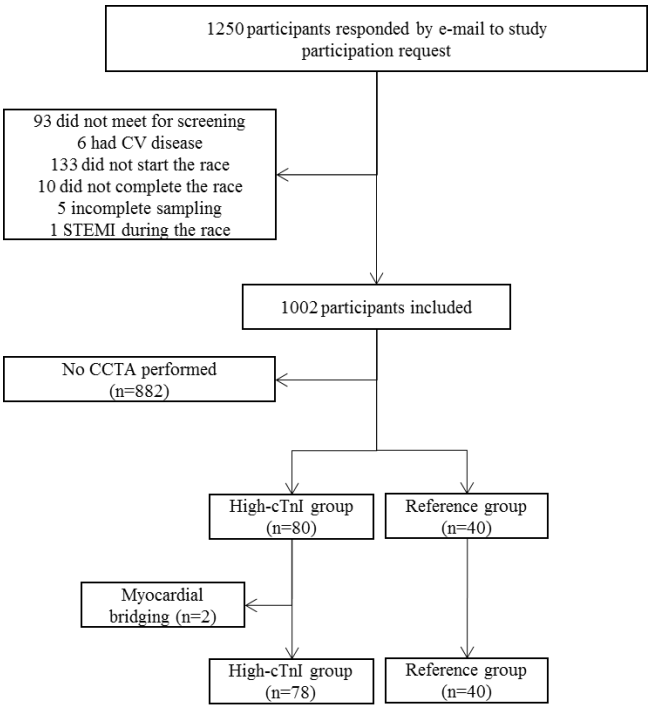


Figure 8: NEEDED 2014 study recruitment. Paper 2 includes an analysis of the 1002 participants that were included in total. Paper 3 includes an analysis on the High-cTnI group vs Reference group subjects.

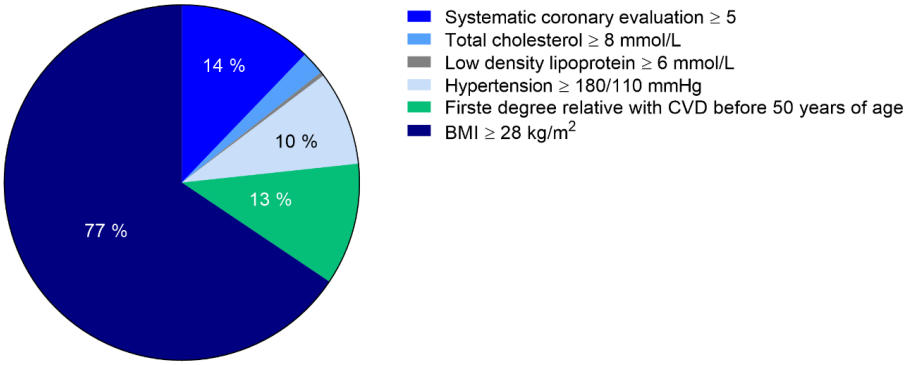


Figure 9: Among the senior athletes (≥35 years of age) in the NEEDED 2014 cohort, 238 subjects had at least one CV risk factor.

9.2 Paper 1

Cardiac troponin I (cTnI) increased from 3.4 (2.1-4.9) ng/L at baseline, to a maximal of 69 (42-98) ng/L at 3 hours after the race (Figure 10). None of the included subjects reported symptoms suggestive of CVD after the race.

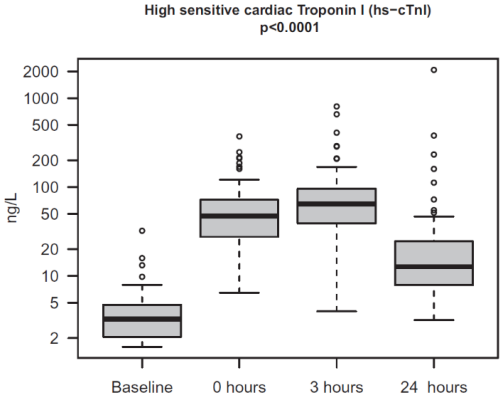


Figure 10: Rise and fall of cTnI in the NEEDED 2013 cohort (n=97).

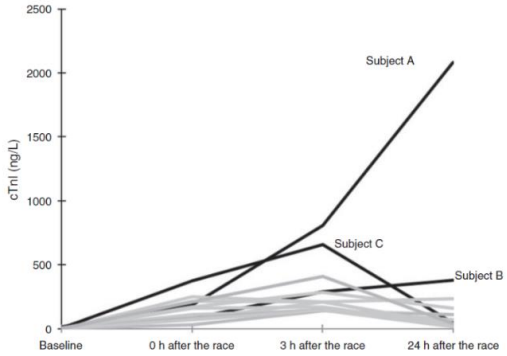


Figure 11: 13 subjects were assessed by CCTA or conventional coronary angiography in the NEEDED 13 study. Subject A had an obstructive lesion in the left anterior descending artery, and an occluded right coronary artery, subject B had obstructive coronary lesion in the circumflex artery, while subject C had a plaque rupture in the left descending artery.

CCTA was obtained in the 13 subjects with the highest cTnI values following the race, and revealed obstructive coronary artery disease in two subjects (Subjects A and B), and a plaque rupture in one subject (Subject C, Figure 11). These three subjects were among the four with the highest cTnI values following the race. These findings suggested that subjects with subclinical coronary artery disease might have an excessive cTnI increase after strenuous exercise, as compared with subjects with no cardiac obstruction.

9.3 Paper 2

Distributions of cTnI and cTnT values at baseline, and at 3- and 24 hours after the race are shown in Figure 12. At 3 hours after the race, 84 % of subjects exceeded the 99th percentile of the cTnI assay (26 ng/L), and 92 % of subjects exceeded the 99th percentile of the cTnT assay (14 ng/L). At 24 hours after the race 18 % (cTnI) and 30 % (cTnT) still had values above the 99th percentile.

In this study, bivariate and multiple regression models identified shorter race duration and higher systolic blood pressure at baseline as consistent significant predictors of the exercise-induced cTn response.

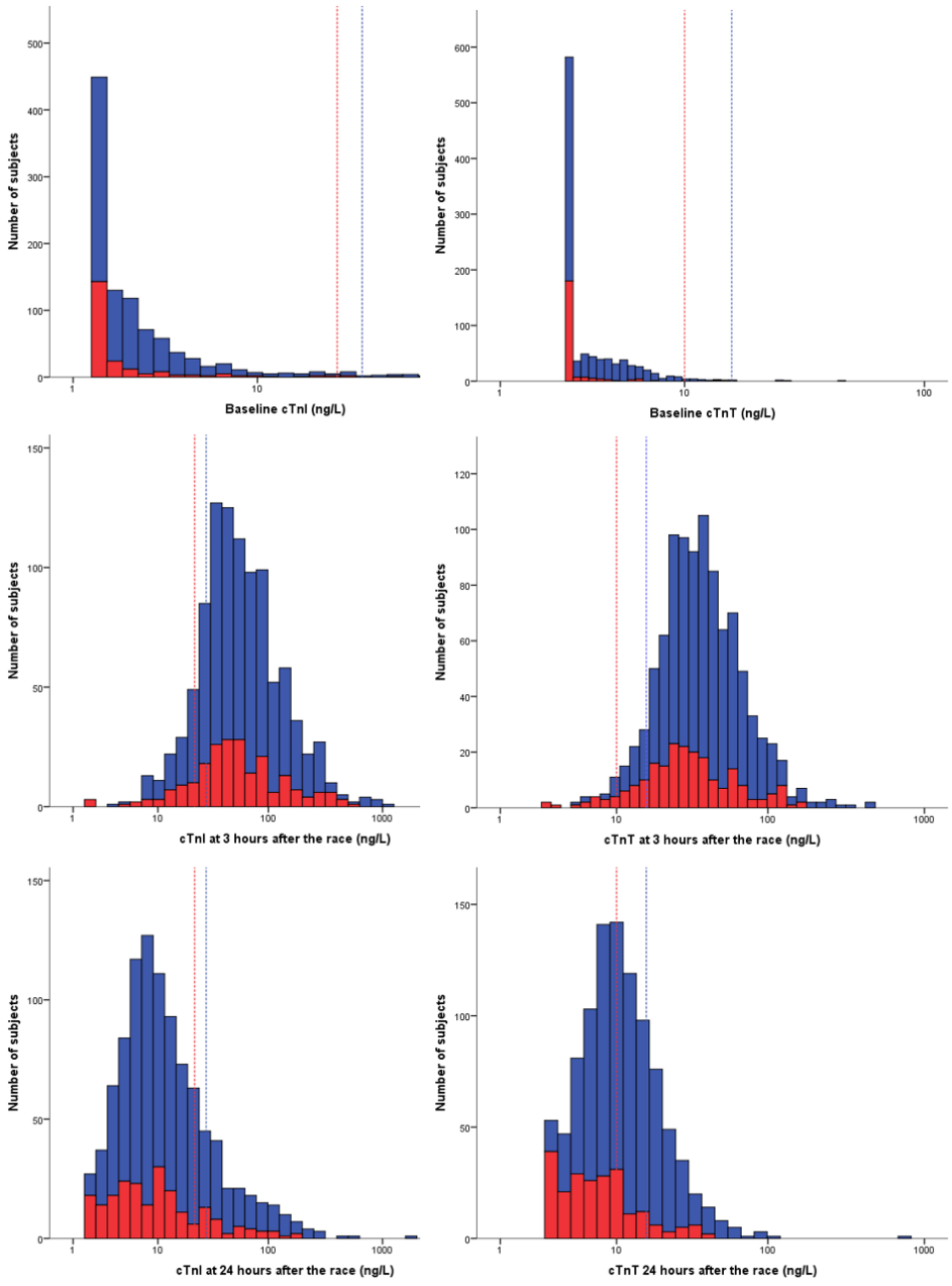


Figure 12: Distribution of cTn at baseline, and at 3- and 24 hours after the race, red indicates female subjects; blue male subjects. Dotted lines represent the sex-specific 99th percentile).

9.4 Paper 3

The 80 subjects with the highest cTnI values at either 3- or 24 hours after the race were examined by CCTA. A reference cohort of 40 subjects with moderately elevated cTnI after the race were also evaluated by CCTA. Baseline characteristics were similar between the two groups, with a mean age of 45±11 years in the High-cTnI group and 46±11 years in the Reference group.

In total, 80 of the 120 subjects had normal coronary arteries, two had myocardial bridging, and 39 had CAD (one of whom also had a myocardial bridge, and as such, she is counted twice).

Obstructive CAD was present in 8 subjects (10 %) in the High-cTnI group, and in one subject (2.5 %) of the Reference group. These 9 subjects had significantly higher cTn values at 24 hours after the race, but not at 3 hours after the race (Figure 13).

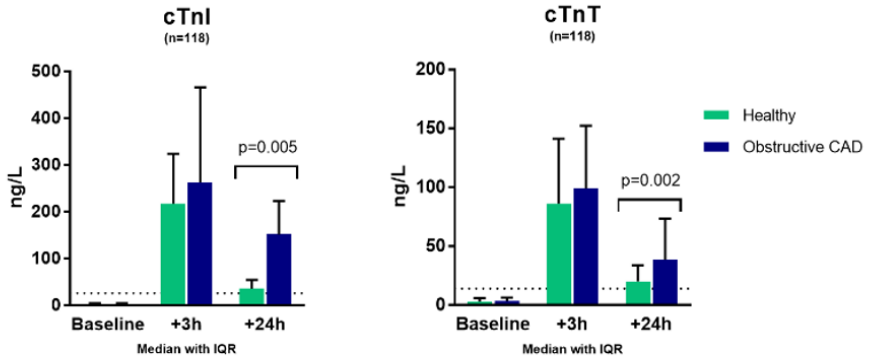


Figure 13: Cardiac troponin I (cTnI) and cardiac troponin T (cTnT) concentrations in subjects assed by coronary computed tomography angiography (n=118). Green columns represent subjects without coronary artery obstruction (n=109). Blue bars represent subjects with obstructive coronary artery disease (CAD, n=9). The two subjects with myocardial bridging are not included. The dotted horizontal line represents the 99th percentile of each assay.

9.5 Paper 4

C-reactive protein (CRP) increased from 0.9 (0.5-1.8) mg/L at baseline to 11.6 (6.0-17.5) mg/L at 24 hours after the race ($p<0.001$). In this study, a significant association with physical fitness and the exercise-induced CRP response was identified, independent of age, sex and body mass index (BMI, Figure 14). A significant association with CRP 24 hours after the race and creatine kinase, a marker of muscular injury, was also identified.

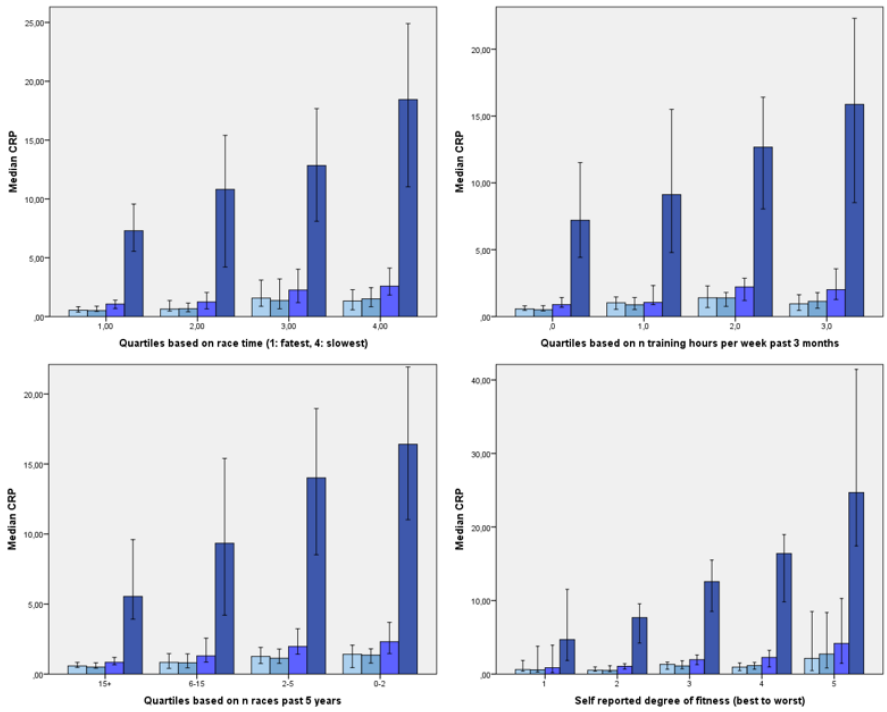


Figure 14: Mean CRP levels before, immediately after the race, 3 hours after the race and 24 hours after the race in subjects with different fitness levels. A: Subjects are parted into quartiles base don race duration from fastest to slowest, B: Subjects are parted into quartiles based upon hours of training per week the 3 months leading up to the race, C: Subjects are parted into quartiles based upon number of previously completed races over the past 5 years, D: self-reported fitness (1: Very good, 2: good, 3: average, 4: below average, 5: poor).

There was no association between the exercise-induced CRP, cTnI and B-type natriuretic peptide response following the race. Also, no association between exercise-induced CRP increase and clinical events at one week after the race was identified.

10. Discussion

Cardiac troponins are sensitive markers of myocardial injury. C-reactive protein is a marker of global inflammation. Both markers are strongly associated with future CV events, and both markers increase following exercise. In this thesis, the physiological cTn and CRP increase is described. For a subset of subjects with underlying obstructive CAD, a potential pathological cTn increase was identified.

10.1 Underlying mechanisms of exercise-induced cTn increase

Cardiac troponins increase due to necrosis of cardiomyocytes. Other mechanisms of cTn increase, i.e. apoptosis or cell wounds, are still poorly understood, particularly in the setting of exercise.

Necrosis of cardiomyocytes is an unlikely explanation for exercise-induced cTn increase due to the low regeneration rate of cardiomyocytes in humans, and the frequency in which cTn increase with exercise is described (116). This is supported by mechanistic data by Weil et al., who found that an increase in preload was associated with a transient cTn increase in the absence of ischaemia (181). It has been suggested that exercise-induced cTn increase due to leakage of loosely bound cytoplasmic cTn by blebbing or increased membrane permeability, however, more research in this area is needed in order to conclude on the underlying mechanisms of exercise-induced cTn increase (116,118). Studies on cTn fragments might be one such avenue in order to understand these underlying mechanisms. One small study found that cTnT only existed as secondary fragments in marathon runners (95). These findings need to be confirmed in larger cohorts, however, there are still analytical difficulties with these methods.

The Papers in this thesis have not directly explored the underlying mechanisms of exercise-induced cTn increase. The high prevalence of cTn increase above the 99th percentile of the assays that was found in Paper 2, however, underscores the unlikeliness of necrosis as the primary mechanism of exercise-induced cTn increase

in the majority of participants. Further work on cTn characterisation of the NEEDED samples is currently ongoing.

10.2 Kinetics of exercise-induced cTn

Several studies have sampled cTn several times following exercise to describe the cTn kinetics. The landmark study by Middleton et al. studied the kinetics of cTnT release in relation to a marathon in 9 subjects. In this study, a biphasic cTnT release was noted, with the first peak during the marathon, and the second peak at 6-24 hours after the marathon (124). According to a review article by Gresslien et al. there is evidence to support a peak in cTnT at 2-5 hours post-exercise, and a peak in cTnI at 3-6 hours post-exercise (116). The underlying evidence of this statement, however, is rather weak, and based primarily on very small and selected cohorts. None of the citations for this statement included studies solely on cycling events.

Scherr et al. sampled cTnT prior to a marathon, and immediately- , 24 hours- and 72 hours after the marathon (168). They found a peak in cTnT immediately after the race, however, with no sampling between 0-24 hours post-race, this study did not provide a definite time of peak for exercise-induced cTnT elevation (168). The study by Herrmann et al. included 46 marathon runners, and sampled cTnI and cTnT at baseline, and at 15 minutes and 3 hours post-race (123). They found that cTn continued to increase from 15 minutes post-race to 3 hours post-race in most subjects, supporting the statement by Gresslien et al (116). Similar findings were presented by Sharhag et al., who also included a subgroup of cyclists (122).

At the time we designed the NEEDED study, there was scarce evidence on the expected kinetics of cTn in relation to exercise in general, and to prolonged cycling in particular. In paper 1, we confirmed that cTnI was higher at 3 hours post-exercise than immediately after exercise. The cTnI levels had not completely normalized within 24 hours following the race. Due to logistical challenges, adding more sampling time-points to the protocol was not feasible. Additional sampling time points would have added valuable information about the best time of sampling for

peak cTnI. To achieve the best degree of comparability between the pilot-study and the main study (NEEDED 2014, papers 2-3), the same time-point of 3 hours post-race was used for the main study.

10.3 Predictors of exercise-induced cTn elevation

Gresslien et al. reviewed the literature concerning cTn increase and exercise between 1987 and 2015, and included a total of 145 studies (116). There are several methodological difficulties in comparing these 145 studies, particularly due to the use of different cTn assays, and different protocols of cTn sampling. Most of these studies only sampled cTn immediately after exercise, and many only included male subjects. Also, only 8 of these studies included more than 100 subjects (117,168,182-187).

Of these 8 larger studies of > 100 included subjects, two were performed on low-intensity or duration exercise (184,187), and might therefore not be fully comparable to the other 6, which were conducted on 21-42 km running events (117,168,182,183,185,186). Fortescue et al. (n=482) was the largest investigation prior to the NEEDED study, and was the only one of the 8 studies of > 100 subjects which studied both cTnI and cTnT. This study, however, only sampled cTn before and immediately following a marathon. Also, data collection was performed in 2002, and older cTn assays were used. In statistical analyses, subjects were assessed as above or below the 99th percentile cut-off, and the authors found that runners with troponin increase were younger, and more likely to be women. Runners with no prior marathon experience were also more likely to have a post-race cTn above the cut-off. Shalen et al. used very similar methodology, and they also found that no prior race experience was associated with cTn increase above the 99th percentile, but in contrast, they also identified higher age and creatinine increase as predictors in their analysis (185). Jassal et al. assessed cTn shortly after a marathon, and concluded that less running experience was associated with higher cTn values (183). Mingels et al. found that a longer running distance was associated with higher cTn increase (187).

Scherr et al. is the largest study prior to the NEEDED study which presented a linear regression analysis to determine the predictors of the exercise-induced cTn. They only presented data on cTnT increase from baseline to immediately after the race, but found that higher IL-6, lower age, higher body fat and higher increase in h-fatty acid binding protein were associated with the cTnT increase (R^2=0.29). The study by Eijsvogels et al. on prolonged walking also presented a linear regression analysis, and found that higher age, CV pathology and walking speed were significant predictors of cTn elevation (R^2=0.11) (184).

Another slightly smaller study by Eijsvogels et al. assessed predictors of the cTn release immediately after a marathon in a subgroup of 67 athletes. They found that lower age and longer exercise duration were significant predictors (R^2=0.093) (188). Mingels et al. included 85 subjects and also sampled immediately after the race (167). In their multiple regression analysis, higher age and lower running experience were significant predictors. The largest study on exercise-induced cTn in relation to cycling (n=91) also sampled cTnI immediately after the race (189). This study only presented bivariate correlations between different predictors and cTn (189).

The studies by Scherr et al., Eijsvogels et al., and Mingels et al., are some of the studies who presents the most reliable data on predictors of the exercise-induced cTn increase, however, they are all limited by sampling at a time where cTn is expected to continue to increase, and by their relative low number of included subjects (167,168,188). The three studies also did not include the same variables into their models. Two of them found that age was inversely associated with increased exercise-induced cTn (168,188), while one study found a positive association (167). Most of the presented studies agree that less training experience is also associated with a higher degree of cTn increase post-exercise.

Paper 2 in this thesis details the conflicting evidence surrounding different variables that are believed to be associated with the exercise-induced cTn increase. In this paper, comprehensive multiple regression analyses are presented for the largest cohort to date (n=1002). Our findings are presented at baseline, 3 hours- and 24 hours

post-race, and for both cTnI and cTnT. Multiple regression analyses are also presented for delta cTn values in the Supplementary Appendix, with very similar results as presented in the manuscript.

Contrary to other studies, we found that the major predictors of exercise-induced cTn increase were lower race duration and higher systolic blood pressure. It is important to note that systolic blood pressure was not included into several of the linear regression models in the previous studies (167,168,188).

Age was not a consistent predictor in the multiple regression models. Also, training experience was not found to be significantly associated with the cTn increase. In fact, one could argue that subjects who are able to finish the race faster have a higher physical fitness level than subjects who cycled slower. This is supported by the findings in Paper 4, where there were good correlations between race duration, self-reported physical fitness, number of previous competitions over the past 5 years and exercise volume prior to the race. Race duration, however, also reflects technical skills and exercise intensity, which needs to be considered when interpreting this parameter. Subjects with low race duration might have spent a large percentage of the race at a very high intensity level. High intensity work and increased peripheral resistance (systolic blood pressure) are both parameters that reflect cardiac work load.

Body composition has been evaluated in several prior studies, and in a meta-regression analysis, body weight was positively correlated with increased exercise-induced cTn levels (125,190). In our study, however, body composition had inconsistent correlations with the exercise-induced cTn increase. Other CV risk factors, like cholesterol levels, family history of premature CV disease or the Framingham Risk Score was also not significantly associated with the exercise-induced cTn increase. Subjects with at least one CV risk factor did, however, have slightly higher cTn levels 24 hours post-race as compared with subjects with no risk factors ($p<0.01$).

Paper 2 of this thesis provides important and novel insight into the clinical variables that are associated with exercise-induced cTn increase. It is important to note the low

coefficient of variance for these models. This suggests that unknown factors are of major importance in this process, and further research is needed.

10.4 Cardiovascular disease in the NEEDED 2014 cohort

In the NEEDED 2013 cohort, only a limited number of subjects were assessed by CCTA. In the NEEDED 2014 study, however, 120 subjects were examined by CCTA. A median CAC score of 0 (0-3) Agatston Units was found for this cohort (n=119, one subject in the High-cTnI group was only assessed by invasive coronary angiography). The CAC scores in this cohort were in line with the study by Merghani et al. (39) and by findings from population-based cohorts (191,192), but lower than that found by Aengevaeren et al., Möhlenkamp et al., and Braber et al (43,50,51). Only 29 % of the 119 subjects assessed by CCTA in Paper 3 had CAC > 0 Agatston Units (males: 33 %, females: 19 %), which is also lower than in the previous studies on recreational athletes. One major reason for the lower prevalence of CAC in our cohort is that the mean age was 45±11 years, contrasting a mean age of 54-57 years in previous studies (39,43,50,51). Also, 26 % of the present cohort were female.

CAC is an indicator of the burden of coronary artery atherosclerosis. CAC, however, lacks the ability to identify luminal stenosis and vulnerable plaques, i.e. plaques with large necrotic cores, and macrophage infiltration in the fibrous cap. Having CAD on CCTA has been found to significantly increase the risk of future CV events (193). Subjects with obstructive lesions (>50 % stenosis) have a higher risk of CV events than subjects with non-obstructive lesions (193,194). Subjects with non-obstructive lesions, however, still have a 3-year risk of 1.3-3.7 % of major adverse cardiac events (166,194). Since most findings on CCTA are of a non-obstructive nature, this group is of importance when trying to prevent future CV events. Using CCTA and plaque characterization, Hou et al. found that patients with calcified plaques only had less clinical events than subjects with non-calcified or mixed plaques, suggesting that calcified plaques are a more stable form of CAD (194).

Nine subjects (7.5 %) in the NEEDED 2014 CCTA cohort had obstructive CAD. Braber et al. found that 5.3 % of sportsmen in their cohort had obstructive lesions (50). Merghani et al. reported a 3.3 % prevalence of luminal stenosis > 50 % in their cohort of male and female athletes (39). Mölenkamp et al., and Aengevaeren et al. did not report CCTA data (43,51). Mean age of study subjects in the studies by Braber et al. and Merghani et al. were 55 years, contrasting our cohort with a median age of 45 years of age (39,50). As such, there was a higher prevalence of obstructive CAD in our cohort as compared to these two studies, even with a markedly lower mean age of study subjects and inclusion of female subjects in our cohort. It is likely that this reflects selection of study subjects. The most striking difference in selection of study subjects between NEEDED 2014 study (Paper 3) and Braber et al. and Merghani et al., is that 66 % of our subjects were selected based on highly increased exercise-induced cTnI. Eight of the nine subjects (89 %) with obstructive CAD were identified within the High-cTnI group.

10.5 Cardiac troponins and cardiovascular disease

Increased levels of cTn has been associated with worse outcomes in population based studies (112,113), as well as for subjects with CVD (195), arrhythmias (78), non-cardiac surgery (80), end-stage renal disease (76) and stroke (79). The long-term outcomes of subjects with exercise-induced cTn have been evaluated in two studies, both published after the initiation of the NEEDED project. Möhlenkamp et al. found no association between CVD and exercise-induced cTnI in 74 marathon runners (82). In contrast, Aengevaeren et al. (n=725) found a highly significant association between exercise-induced cTnI above the upper reference limit and CV events in their cohort of older subjects performing 30-55 km of walking (83).

Since there seems to be an association between cTn and future CVD in population-based studies, an association between cTn and the degree of CAD is expected. This was assessed by Reckord et al., who found higher cTn concentrations in subjects with more severe CAD on CCTA (196). A small study by Rusnak et al. also found that

subjects with mixed plaques had higher cTn levels than subjects with no CAD or with calcified or non-calcified plaques (197).

10.6 Obstructive coronary artery disease in relation to exercise-induced cTn increase

In Paper 1, obstructive CAD was identified in two subjects, while one subject had a plaque rupture. In Paper 3, nine subjects had obstructive CAD. Subjects with obstructive CAD had significantly higher cTnI and cTnT levels 24 hours after the race, as compared with subjects with normal coronary arteries or non-obstructive CAD (cTnI: 151 (72-232) vs 36 (19-82) ng/L, p=0.005, cTnT: 39 (35-55) vs 20 (14-31) ng/L, p=0.002). cTn was found to be able to discriminate the subjects with obstructive CAD from the rest of the cohort, with AUC of 0.79 (95 % CI: 0.61-0.96), p=0.005 for cTnI and 0.82 (0.70-0.94), p=0.002 for cTnT (Paper 3).

The difference in cTn kinetics between subjects with obstructive CAD and subjects without coronary obstruction could be related to demand ischaemia and a degree of cardiomyocytes necrosis. As described in chapter 10.3, physiological exercise-induced cTn tends to peak at 2-6 hours post-exercise, and then normalize at 24-72 hours post-exercise. This contrasts the later rise in cTn levels seen following myocardial infarction, where there is a release of structural cTn due to cellular necrosis (100). This hypothesis, however, is difficult to prove with certainty, and more research is needed.

Subject C in Paper 3 had a cTnI profile of 1.7 ng/L-28 ng/L-8 ng/L. This subject was a Reference group subjects who had an obstructive lesion in the left anterior descending artery with an FFR of 0.78, obtained almost a year after the race. We cannot be certain that this subject had an obstructive lesion at the time of cTn sampling. Subject I also had a cTnI profile that might be lower than expected with a 90 % obstruction of the LAD (1.6 ng/L-263 ng/L-41 ng/L). This subject was a 66-year old female who used 4.9 hours to finish the race (above the 75th percentile for the entire cohort of the NEEDED 2014 study). The present study did not mandate the

use of heart rate monitors to assess work during the race, and as such, we cannot be certain that this subject performed sufficient high-intensity work during the race to induce demand ischaemia. The exact association between cardiac work and cTn increase needs further research. Also, as described in chapter 10.4, there are large individual differences in exercise-induced cTn not accounted for by commonly used clinical parameters.

There was no statistically significant difference in prevalence of obstructive CAD between the High-cTnI group and the Reference group. The study was underpowered to prove a difference at the observed level. When the prevalence of obstructive CAD in the Reference group (2.5%) was extrapolated to the cohort of subjects where CCTA was not obtained (n=882), a highly significant difference between the High-cTn group and the rest of the cohort was observed (OR 4.28 [1.85-9.87], p=0.002). However, we cannot be certain that the prevalence of obstructive CAD in the Reference group reflects the true prevalence in the remaining subjects with a low or moderately elevated cTn following the race. We therefor chose to omit this finding from the manuscript.

Paper 3 is the first study to demonstrate exercise-induced cTn levels in subgroups of patients with normal coronary arteries, non-obstructive CAD and obstructive CAD. The findings suggest a potential role of exercise-induced cTn in determining if an athlete has physiological or potentially pathological response to exercise, however, more research is needed to confirm and extend these findings to different cohorts.

10.7 Non-obstructive CAD in relation to exercise-induced cTn increase

CV events in subjects with vulnerable, non-obstructive plaques are prevalent (198). The association between non-obstructive plaques and exercise-induced cTn increase, however, is uncertain. Möhlenkamp et al. and Paana et al. found no association between CAC score and cTn obtained immediately following a marathon (82,199). This non-significant association between CAC and cTn at 3- and 24 hours was

confirmed in our material. Also, subjects with non-obstructive CAD did not have higher cTn levels as compared with subjects with normal coronary arteries (Table 3). The combined analysis of the CCTA findings from the NEEDED 2013 and 2014 cohorts (Paper 1 and 3) were presented at EuroPrevent 2019 (200). In this presentation, plaque morphology was assessed, and among the 30 subjects with non-obstructive CAD, 20 (67 %) had calcified plaques only. There was no significant difference in cTn kinetics among subjects with non-calcified or mixed plaques as compared to subjects with calcified plaques only. These findings support the hypothesis that a supply-demand mismatch is important for generating a prolonged elevation of exercise-induced cTn, as seen for subjects with obstructive CAD.

High-risk plaques features include low attenuation (0-30 Hounsfield Units), positive remodelling and the Glagov phenomenon, as well as plaques with spotty calcification/napkin ring sign (201). These plaque features have not yet been analysed within the NEEDED cohort.

Table 3 cTnI and cTnT in participants from the combined CCTA examinations from the 2013 and 2014 studies (n=133). Values are presented as median (25^{th}-75^{th} percentile), or as mean (minimum-maximum) for myocardial bridging due to n=2.

	Normal coronary arteries (n=90)	**Non-obstructive CAD (n=30)**	**Myocardial bridging (n=2)**	**Obstructive CAD (n=11)**
cTnI baseline, ng/L	2.3 (2-6)	3.7 (2-7)	7.0 (6-8)	2.8 (2-7)
cTnI +3h, ng/L	224 (150-303)	182 (76-257)	772 (443-1002)	269 (206-808)
cTnI +24h, ng/L	36 (19-82)	36 (19-83)	145 (110-181)	169 (102-380)
cTnT baseline, ng/L	3.0 (3-5)	3.3 (3-5)	3.5 (3-4)	4.5 (3-10)
cTnT +3h, ng/L	85 (60-123)	84 (38-124)	220 (115-325)	100 (59-140)
cTnT +24h, ng/L	21 (14-34)	23 (13-29)	43 (31-54)	44 (38-75)

10.8 Myocardial bridging in relation to exercise-induced cTn increase

Two subjects, both in the High-cTnI group, had myocardial bridging on CCTA. Their characteristics are outlined in Paper 3. As shown in Table 4, both of these subjects had lesions located in the mid left anterior descending artery (LAD). Myocardial bridging with a depth of ≥1 mm might be considered deep (202).

Table 4: Characteristics of the two subjects with myocardial bridging. LAD=Left anterior descending artery.

	Age (years)	**Sex**	**Localization**	**Length (mm)**	**Depth (mm)**
Subject A	27	Male	LAD, segment 7	20	4
Subject B	55	Female	LAD, segment 6	25	3

Myocardial bridging is an intramural course of an epicardial coronary artery. In systole, myocardial bridges might compress blood flow through the tunnelled artery. However, under normal circumstances, approximately 85 % of the coronary blood flow occurs during diastole (203). It is also a very common finding on CCTA, with Dimitriu-Leen et al. reporting that 22 % of low- and intermediate risk subjects who were referred to a CCTA had some degree of myocardial bridging (204). A review on this topic concluded that approximately one in four have a myocardial bridge (203). The frequency and underlying understanding of the coronary blood flow suggest that a myocardial bridge is a physiological phenomenon in most cases. This is also supported by long-term follow-up studies who did not find that myocardial bridging was associated with worse cardiac outcomes (204,205).

Both of our study subjects were asymptomatic during and after the race, and had no findings suggestive of ischaemia on ECG. We decided not to do further testing or follow-up procedures for these two subjects (other than for all the NEEDED study subjects). The current ESC sport cardiology recommendations states that the presence of exercise-induced ischaemia or complex ventricular tachyarrhythmias in subjects

with a myocardial bridge should warrant restriction from participation in competitive sport (level of recommendation: Class IIa, level of evidence C) (206). As such, a maximal exercise test might be indicated in these subjects. A study by Kramer et al. from 1982, however, only identified a positive stress test in 3/25 symptomatic subjects with myocardial bridging identified by coronary angiography (207). The best functional assessment of a myocardial bridge is probably a diastolic fractional flow reserve with the use of dobutamine challenge (203). This is an invasive procedure, which, in our opinion, would not influence the clinical decision-making for these asymptomatic subjects. Our decision is supported by a flow-chart on management of myocardial bridging of the left anterior descending artery proposed by Tarantini et al (203).

Interestingly, subject B also had non-obstructive CAD. Coronary artery plaques are a common finding in subjects with myocardial bridging, most likely due to high systolic wall shear stress upstream from the bridge (203). Subject B, however, had a non-calcified plaque in her right coronary artery, and the area proximal to the myocardial bridge was not affected.

There is limited knowledge on the consequences of myocardial bridging and the risk of sport-related cardiac events. Exercise-induced premature ventricular complexes and non-sustained ventricular arrhythmias have also been described to be more frequent in subjects with myocardial bridging (208), as well as case-reports of myocardial infarction and sudden death (209). It is interesting that both of our subjects had highly elevated cTn following the race, and this might suggest a supply-demand mismatch in these individuals. It is important to acknowledge, though, that cTn elevation does not equal ischaemia, and several other subjects with no myocardial bridge had similarly elevated cTn following the race. Importantly, no adverse clinical events have been identified for these two individuals since the 2014 race.

10.9 Exercise-induced CRP increase

Prior to the NEEDED study, there was uncertainty about the kinetics of exercise-induced CRP, the magnitude of the CRP increase and the predictors of this response. The clinical significance of the exercise-induced CRP response was also unknown. We addressed these topics in Paper 4, and found an 129 % increase in CRP levels 24 hours following the North Sea Race, as compared to baseline levels, $p<0.001$. The magnitude was less than described following marathon-running by Weight et al., (155), but similar as described by Sherr et al. (168). We did not sample after 24 hours following exercise, however, other studies have confirmed that exercise-induced CRP levels decrease at 48-72 hours following strenuous exercise (168,210,211).

In bivariate and multiple regression analyses, physical fitness was significantly inversely associated with the magnitude of the CRP response, assessed as race duration, self-reported fitness level, number of prior endurance exercise competitions during the past 5 years and number of hours per week of endurance exercise training. The present study did not use peak oxygen consumption to determine physical fitness; however, all measures of physical fitness were well correlated with each other.

The correlation between physical fitness and exercise-induced CRP increase might have been anticipated, as less fit subjects have smaller cardiac dimensions, less developed musculature and vasculature and a lower degree of exercise efficiency as compared with highly trained athletes. During acute exercise, contracting viable (i.e. non-damaged) skeletal muscle serve as a secretory organ, secreting pleiotropic molecules collectively called myokines which exerts paracrine and endocrine effects on various target organs. During and shortly following physical activity, IL-6 is released from myocytes causing plasma levels to increase hundred-fold compared with baseline values (153,154). IL-6 serves as an upstream regulator of CRP production in the liver, and Scharhag et al. showed that exercise-induced CRP and IL-6 were strongly correlated ($R=0.68$, $p<0.001$) (212). This association between muscle metabolism and inflammation might also be the reason why an association

between exercise-induced CRP and CK was identified. The correlation between exercise-induced CRP and CK is in line with findings by Strachan et al. (210).

Findings from the present study confirms and extends on prior findings. Liesen et al. found an attenuation of the exercise-induced CRP response following 9 weeks of endurance training; however, this study only included three male subjects (213). Mündermann et al. (n=45) described a relationship between marathon performance and exercise-induced CRP, however they included male subjects only (214). The findings of the present study suggest that the beneficial effects of high degree of physical fitness might not only relate to a favourable modulation of basal inflammation, but also an improved biological response to physical stress.

There are known associations between baseline CV risk factors and CRP, and as such, the influence of CV risk factors on exercise-induced CRP was assessed in multiple regression analysis. At baseline, BMI was significantly associated with the CRP level of the present cohort. The association between BMI and exercise-induced CRP levels, however, was not significant in the present study. This is in line with findings by Mündermann et al. who also did not find an association between BMI and exercise-induced CRP (214). We wanted to assess the relationship between CV risk factors and the exercise-induced CRP response further in the 2014 cohort in order to confirm this finding, however, in this larger cohort, BMI was significantly associated with exercise-induced CRP increase (Figure 15) (215). Baseline LDL levels also had a significant bivariate correlation with exercise-induced CRP in the 2014 cohort (rho=0.12, $p<0.001$). Systolic blood pressure was not associated with exercise-induced CRP increase. As such, it is important to note that in small studies, and particularly studies with homogenous cohorts, certain associations are difficult to assess with certainty.

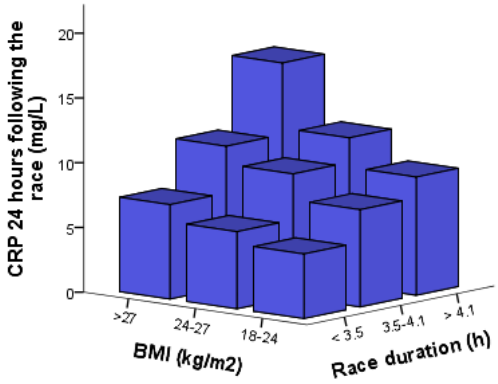


Figure 15: The association between BMI, race duration and the CRP increase during the NEEDED 2014 study (n=1002) (215).

A secondary aim of Paper 4 was to evaluate the association between the exercise-induced CRP, cTnI and BNP after strenuous exercise. No significant relationship was observed. This is in line with findings by Scherr et al., who also did not find an association between CRP and cTnT following a marathon (168). They, however, did not sample cTnT at the expected peak at 2-6 hours following exercise (168). Scharhag et al. assessed the association between CRP and N terminal pro brain natriuretic peptide (NT-proBNP) (212). They found no association between exercise-induced CRP and NT-proBNP levels in their cohort of 14 athletes, suggesting that the exercise-induced increase in NT-proBNP was unrelated to inflammation (212).

In order to assess the significance of the exercise-induced CRP increase, data on infections and well-being at 1-week post-race was collected. Clinical events following the NEEDED 2013 study were defined as self-reported unscheduled healthcare contact (n=3), sick leave or unusual discomfort 1 week following the race (n=9). These subjects had non-significantly increased levels of CRP both prior to the race and 24 hours following the race. These findings are reassuring; however, the small sample-size of this study prevents us to conclude with certainty on the clinical significance of exercise-induced CRP. The association between exercise-induced CRP increase and clinical end events will be further assessed in the 5-year follow-up

study of the NEEDED 2014 cohort, where the primary end-pint is a composite of mortality, myocardial infarction, revascularization, sudden cardiac arrest, heart failure and stroke.

10.10 Other exercise-induced biomarkers

The NEEDED study program measured several cardiac biomarkers. In Paper 1, the kinetics of creatine kinase – myocardial band (CK-MB), creatine kinase (CK), B-type natriuretic peptide (BNP) and creatinine are outlined, all of whom increased significantly from baseline ($p<0.0001$). We also assessed copeptin in the pilot cohort, and did not find an association between the exercise-induced copeptin-response and the subjects with CAD (216). Lastly, we have several other manuscripts currently under construction or consideration, most notably one on exercise-induced changes in lipoproteins, and one on 4-year follow-up of CCTA indices.

10.11 Clinical implications and future perspectives

Endurance exercise training is highly beneficial for good health. High-intensity exercise, however, does carry a small risk of sudden cardiac arrest. Prior research has focused on pre-participation evaluation of athletes in order to determine if a subject it at risk of a sport-related cardiac event. The NEEDED project explores to role of exercise-induced cardiac biomarkers in determining if certain subjects might have a pathological response to exercise, and thereby be at higher risk of future CV events.

Findings from the NEEDED studies are novel in several aspects, particularly due to the use of CCTA in assessing the relationship between CAD and exercise-induced cardiac biomarkers. This is the first study to show that exercise-induced cTn has a prolonged elevation in subjects with occult obstructive CAD, discriminating these subjects from subjects with no coronary obstruction. As such, this finding could potentially lead to the use of post-exercise cTn measurements to identify subjects at risk of a cardiac event. However, the data presented thus far is cross-sectional of nature, and is only valid for this cohort of recreational athletes. Our findings need to

be confirmed in other cohorts, and the long-term clinical significance must be determined.

The NEEDED study also shows that measuring cTn immediately following exercise is insufficient, and that later sampling is necessary to detect peak cTn and to find a difference between subjects with potentially clinical relevant CAD. This is of importance for future studies of similar design.

The overall aim of the NEEDED project is to assess long-term CV health in recreational athletes. As such, follow-up data will be collected at 5-, 10- and 20 years after the NEEDED 2014 study.

Several avenues of research are ongoing within the NEEDED research program. First, characterization of cTn molecules is one area of interest that might increase the understanding of the underlying mechanisms of cTn release in relation to exercise. Second, data regarding the 5-year risk associated with the exercise-induced increase in cardiac biomarkers is currently being collected and analysed. Third, there is still a high degree of uncertainty about the association between cardiac work and exercise-induced cTn increase. This is being assessed as part of the physiological NEEDED 2018 study (n=62). This study also included echocardiographic data on study participants before and after strenuous exercise, including strain analysis and three-dimensional image acquisition. These data might increase our understanding of the association between cardiac function and exercise-induced cardiac biomarkers. Forth, exercise-induced changes in electrocardiograms are being analysed, and will provide us with further understanding of exercise-induced electrical changes in relation to cardiac biomarkers. Lastly, we have two projects planned, one on chronic cTn increase in Olympic athletes, and one on determining the threshold of exercise needed to induce a cTn increase. Further description of other cardiac biomarkers in relation to exercise will also be published.

10.12 General limitations

The findings presented in this thesis are based on observational data, and subjects were recruited based on their participation in the North Sea Race. This generated a pool of study subjects that were predominantly male, and of Caucasian ethnicity. The included subjects were probably more physically active than the general population. Most of the included subjects, however, were not masters athletes, and we have used the term “recreational athlete” in order to describe the current study populations, the word that in our opinion most closely mimics the Norwegian term “mosjonist”.

Other selection biases might also have affected the results, such as the interest in a comprehensive cardiac work-up, a motivational factor that was present in > 70 % of our study subjects (see chapter 8). Attrition bias should also be mentioned as a general limitation of this study, as several participants, both in the pilot and the main study, registered for the study, but did not complete the race or data sampling. These subjects were not included in the analyses.

The quality of data collection was generally very high, with trained nurses and bioengineers performing most of the procedures. The physiological parameters that were obtained several times were generally very well correlated, with Spearman’s rank correlation coefficient > 0.9. However, with such a large data acquisition, there are likely to be some errors, both of a human nature and due to analytical difficulties with analysing > 3000 batches of blood in a 72-hour period. Blood samples that were found to be outliers underwent quality testing.

In both the pilot study and the 2014 main study, blood samples were acquired at baseline and within 24 hours following the race. This precludes us from determining if later exercise-induced changes in cardiac biomarkers might be of importance. Also, due to the large sample-sizes, physiological data such as peak O_2 and echocardiographic assessments were not collected. Questions or analysis to identify possible doping among our study subjects were also not included as part of the study protocol.

The protocol for the main study was based on findings from the pilot study, and reflects the evolution of knowledge during this project. As we did not have the resources to include all study subjects for a CCTA, we had to choose which participants to include for the CCTA sub-studies. In the main study, we chose to include the 80 subjects with the highest cTn at any time after the race. This decision was based the strong association between cTn increase and risk of CAD in a clinical setting, findings from the pilot study, and safety consideration for subjects with highly elevated cTn.

The major limitation of this thesis pertains to Paper 3. As an amendment to the study protocol, a Reference group of study subjects (n=40) with moderately increased cTn was included for the CCTA study. CCTA was obtained later for these individuals, some almost a year after the race. The Reference group was added particularly due to work on the initial publication (Paper 1). We hypothesized that subjects with higher exercise-induced cTn levels were at higher risk of having underlying CAD. The Reference group was supposed to prove or disprove this hypothesis. The limited size of the Reference group, however, precluded us from drawing a final conclusion. Sample size calculation was very difficult, as no comparable studies had been published. Based on the pilot study, a prevalence of CAD between 25-37.5 % in the High-cTnI group was expected. We acknowledge that, retrospectively, this estimate was too high to be readily transferred to the main study. The observed difference between a 2.5 % and a 10 % prevalence of obstructive CAD in the Reference group vs the High-cTnI group was not statistically significant. The present study was underpowered to prove a significant difference at this level. An underpowered study increases the risk of type II error (false negative results). As such, we should interpret this non-significant difference with caution.

In order to design a future study to find a difference between High-cTnI and Reference group subjects, 162 subjects would have to be included in both groups to have a power of 80 %. In order to identify 162 High-cTnI subjects with the same methodology as we used, approximately 2000 recreational athletes would have to be included in total. This underscores the difficulties in studying rare occurrences.

Lastly, multiple testing was used in all Papers, particularly in Paper 2 and 4. As this work was explorative, correction for multiple testing was not applied. Correcting for multiple testing might have been indicated, however, it also increases the risk of type II errors (false negative results) (217).

10.12.1 Specific limitations of Paper 1

The pilot study was a hypothesis generating study. The study only obtained coronary angiography to assess the athletes with the highest cTnI levels. Only three subjects with significant CAD were identified. As such, it was difficult to assess with certainty if maximal cTnI values were superior in predicting risk of CAD as compared to cTnI values at 24 hours post-race. The sampling time immediately after the race, however, was not found to identify subjects with underlying CAD, and as such, this sampling point was omitted for the main study (due to logistical concerns with two sampling times on one day on >1000 subjects). Also, no echocardiographic or ischemic assessments were obtained. Finally, a cut-off value of cTn is presented in this paper, based on very preliminary data. This cut-off value should be viewed with great caution based upon the methodology and sample size of this study.

10.12.2 Specific limitations of Paper 2

The major limitations of this study relate to the lack of mechanistic data such as echocardiographic and ischemia assessment. This was not possible due to the large sample size. As such, the impact of long-term cardiovascular adaptations to physical activity, i.e. athlete's heart, on exercise-induced cTn increase was not assessed.

The number of female subjects included in this study was much lower than the number of male subjects, reflecting the sex distribution of the North Sea Race participants. This should be considered when interpreting the results on sex-specific cTn results.

Heart rate data was based upon self-reported sport watch measurements, and was only available for a subset of subjects. Data variability between different brands was not considered.

10.12.3 Specific limitations of Paper 3

As discussed above, the Reference group was added to the study protocol after the race. This provided logistical and methodological challenges, and complicated the interpretation of the results. It also underscored the fragility of our observations: One study subject in the Reference group had obstructive CAD, and the FFR of his lesion was 0.78, obtained almost a year after the race. If this subject had not had obstructive CAD, the difference between the prevalence of obstructive CAD in the High-cTnI group and Reference group would have been 0.05 (8/78 vs 0/40, p=0.05), however, the fragility index would have been 1. As such, the findings from this study on the prevalence of obstructive CAD should be interpreted with caution.

Due to logistic challenges with the large sample size of the original study (n=1002), blood sampling was limited to 24 hours before and 3 and 24 hours after the race. The diagnostic potential of later cTn sampling could not be assessed in the current study. Also, no mechanistic data such as echocardiographic and ischemia assessment was obtained as part of the study.

10.12.4 Specific limitations of Paper 4

The CRP response in this study was modest. No determination of peak O_2 consumption was obtained in order to confirm findings regarding physical fitness. We did not assess IL-6 or other upstream markers of inflammation in this study. The number of clinical events were small. Our assessment of the relationship between CRP and clinical endpoints should therefore be interpreted with caution. Long-term clinical outcomes associated with the exercise-induced CRP response is currently being assessed as part of the 5-year follow-up of the 2014 study subjects.

11. Conclusions

Prolonged strenuous physical exercise induces a highly significant increase in biomarkers associated myocardial injury (cTn) and inflammation (CRP) during the first 24 hours following strenuous exercise. The time-dependent changes in these cardiac biomarkers were assessed, and predictors of the exercise-induced cTn and CRP increase were identified. Lastly, the relationship between cTn and CAD was studied, with the identification of a potentially pathological pattern of prolonged cTn elevation for subjects with obstructive CAD.

11.1.1 Paper 1

Cardiac troponin I levels increased significantly from baseline, with maximal values at 3 hours after the race.

Three subjects had subclinical CAD. These were among the four with the highest exercise-induced cTn values after the race.

11.1.2 Paper 2

This large-scale study confirmed that 84-92 % of subject had cTn values above the 99th percentile of the cTn assay 3 hours after the North Sea Race. At 24 hours after the race 18-30 % still had cTn values above the 99th percentile.

Systolic blood pressure and race duration were consistent predictors of the exercise-induced cTn response, both at 3- and 24 hours after the race. These variables were more important than previously reported predictors, such as body mass index, age, sex or training experience.

11.1.3 Paper 3

Cardiac troponin levels 24 hours following strenuous exercise were higher in asymptomatic subjects with obstructive CAD compared with subjects with no coronary artery obstruction, but there was no difference in cTn levels at 3 hours after strenuous exercise. These findings suggest a potential pathological pattern of prolonged elevation of cTn elevation in subjects with obstructive CAD.

11.1.4 Paper 4

CRP increased significantly from baseline, with maximal values measured at 24 hours after the race. Physical fitness was independently and inversely associated with the exercise-induced CRP response. This suggest that the beneficial effects of high degree of physical fitness might not only relate to a favourable modulation of basal inflammation, but also an improved biological response to physical stress. There was no association between the exercise-induced CRP and cTn.

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Race duration and blood pressure are major predictors of exercise-induced cardiac troponin elevation

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ABSTRACT

Background: The underlying mechanisms of the exercise-induced increase in cardiac troponins (cTn) are poorly understood. The aim of this study was to identify independent determinants of exercise-induced cTn increase in a large cohort of healthy recreational athletes.
Methods: A total of 1002 recreational cyclists without known cardiovascular disease or medication, participating in a 91-km mountain bike race were included. Median age was 47 years and 78% were males. Blood samples were obtained 24 h prior to, and 3 and 24 h after the race.
Results: Cardiac TnI concentrations increased markedly from baseline [1.9 (1.6–3.0) ng/L] to 3 h after the race [52.1 (32.4–91.8) ng/L], declining at 24 h after the race [9.9 (6.0–20.0) ng/L]. Similarly, cTnT increased from baseline [3.0 (3.0–4.2) ng/L] to 3 h after the race [35.6 (24.4–54.4) ng/L], followed by a decline at 24 h after the race [10.0 (6.9–15.6) ng/L]. The 99th percentile was exceeded at 3 h after the race in 84% ($n = 842$) of subjects using the cTnI assay and in 92% ($n = 925$) of study subjects using the cTnT assay. Shorter race duration and higher systolic blood pressure (SBP) at baseline were highly significant ($p < 0.001$) independent predictors of exercise-induced cTn increase both in bivariate and multivariable analysis. The age, gender, body mass index, training experience and cardiovascular risk of participants were found to be less consistent predictors.
Conclusion: Systolic blood pressure and race duration were consistent predictors of the exercise-induced cTn increase. These variables likely reflect important mechanisms involved in the exercise-induced cTn elevation.
Trial registration number: NCT02166216 https://clinicaltrials.gov/ct2/show/NCT02166216.

1. Introduction

The "Fourth definition of myocardial infarction" defines a rise and fall pattern of cardiac troponin (cTn) above the 99th percentile as myocardial injury [1]. However, following strenuous exercise there is a rise and fall pattern in cTn in healthy subjects without evidence of irreversible myocardial impairment [2]. Most researchers therefore consider the cTn increase in relation to exercise to be a physiological response [2–5]. The underlying mechanisms and determinants of the exercise-induced cTn increase in healthy individuals are poorly understood. It has been hypothesized that cTn can be released due to reversible myocyte injury and stretch-induced apoptosis, or increased membrane permeability with leakage of loosely bound cTn [2,6]. Exercise-induced troponin increase has also been thought to be due to increased wall tension and ventricular strain caused by volume overload, neuro-hormonal stimulation and/or reversible ischaemia due to increased myocardial energy

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demands [2,7]. Several studies have attempted to identify predictors of the exercise-induced cTn release; however, most of these studies are small, sampled cTn only immediately after exercise or used older cTn assays. Findings from these studies are conflicting, both regarding the influence of age, gender, blood pressure, body composition, training experience and the influence of cardiovascular risk factors [2,4,8–13].

In this large-scale prospective observational study, the aim was to identify the most important predictors associated with the cTn response following strenuous exercise, using high-sensitivity cTnI and cTnT assays.

2. Methods

2.1. Design and study population

This prospective, observational biomarker-study included recreational cyclists ≥16 years of age, residing in Norway without any previous or known CV disease. Subjects were excluded if they reported any CV symptoms, CV treatment or disease (including coronary artery disease, stroke, diabetes mellitus or hypertension requiring treatment). All electrocardiograms (ECGs) were interpreted by experienced cardiologists, and participants were excluded if the ECG had signs of underlying CV disease: Q-waves (>3 mm in depth or > 40 ms in duration in two or more leads except III, aVR and V1), T-inversions (>1 mm in depth in two or more leads in V2-6, II and aVF, or I and aVL), left bundle branch block or atrial or ventricular tachyarrhythmias. Subjects were excluded from this analysis if they did not complete all study assessments. The study was approved by the Regional Ethics Committee (REK 2013/550), and complies with the Declaration of Helsinki. All participants signed informed consent forms prior to enrolment into the study. In total, 1002 participants were included in the present analysis (Supplementary Fig. 1).

2.2. Data collection

An extensive logistic system was developed to allow a comprehensive 30 min assessment of each of the >1000 study subjects 24 h before the race, and at 3 and 24 h following the race (Supplementary Fig. 2). The assessments included ECG, blood pressure measurements, body weight and blood sampling. Detailed clinical information was obtained 5 times by digital questionnaires (Adobe FormsCentral, Adobe Systems Software Ireland Ltd., Ireland). Start- and finishing times were recorded for all participants by the organizer of the race. Subjects reported heart rate data from personal sport watches if available. Age adjusted maximal heart rate was calculated by the formula: $HR_{max} = 208–0.7 * age$ [14].

2.3. Blood samples

Venous blood samples were drawn from the antecubital vein. Cardiac TnI in serum was analyzed within 24 h at Stavanger University Hospital on an Architect i2000SR using the high-sensitive cTnI STAT assay from Abbott Diagnostics (Abbott Diagnostics, IL, USA). Frozen samples were transported on dry ice to Haukeland University Hospital, Bergen, and cTnT was analyzed using a high-sensitivity cTnT assay on Cobas e601 (Roche Diagnostics, Switzerland) in serum that had not been previously thawed. The cTnI assay has a limit of detection of 1.6 ng/L, and the overall 99th percentile of the assay is at 26 ng/L (females: 22 ng/L, males 28 ng/L). The cTnT assay had a limit of blank of 3 ng/L, and a 99th percentile of 14 ng/L (females: 10 ng/L, males 16 ng/L) [15]. Subjects with cTnI values ≤ 1.6 ng/L (n = 403 at baseline, n = 0 at 3 h, n = 13 at 24 h) were given the value 1.6. Subjects with cTnT values below 3 ng/L (n = 582 at baseline, n = 2 at 3 h, n = 31 at 24 h) were given the value of 3 ng/L.

2.4. Statistical analysis

Normally distributed variables are reported as mean ± SD, while continuous variables with markedly skewed distributions are reported as median and (25th percentile - 75th percentile). The Shapiro-Wilk test was used to test for normality. A two-tailed p-value of <0.05 was considered significant. Bivariate correlations between the cTn assays and between cTn and variables previously suggested to be associated with exercise-induced cTn release was assessed at baseline, 3- and 24 h after the race using Spearman's rank correlation. Multiple linear regression analysis was used to identify predictors of the cTn response to exercise at each sampling time-point, using a backward variable elimination procedure. The following variables were included in the models: gender, age, resting heart rate, baseline systolic- and diastolic blood pressure, low density lipoprotein (LDL), estimated glomerular filtration rate ($eGFR_{CKD-EPI}$), body mass index (BMI), race duration, Framingham risk score and baseline cTn values. Residual plots were deemed satisfactory after ln-transformation of the dependent variables. Additional multiple regression analysis was performed in the cohort who reported data from personal sport watches (n = 551), and for delta cTn values, including the same variables as mentioned above. For categorical variables, difference in cTn was assessed by the Mann-Whitney *U* test. For statistical analyses, the statistical software programs SPSS version 24 and GraphPad Prism 7 were used.

3. Results

Participants were 46.8 (40.1–52.6) years old, 78.2% were male (Table 1). Race duration was 3.7 (3.4–4.2) hours. None of the subjects included in this study reported CV symptoms during or following the race.

3.1. Cardiac troponin kinetics

The distributions of cTn values at baseline and at 3- and 24 h after the race are shown in Fig. 1. At baseline, a total of 40.2% (cTnI) and 58.1% (cTnT) had cTn values below the limit of detection (Table 1, Fig. 1). Cardiac TnI concentrations increased markedly from baseline [1.9 (1.6–3.0) ng/L] to 3 h after the race [52.1 (32.4–91.8) ng/L], declining at 24 h after the race [9.9 (6.0–20.0) ng/L]. Similarly, cTnT increased from baseline [3.0 (3.0–4.2) ng/L] to 3 h after the race [35.6 (24.4–54.4) ng/L], followed by a decline at 24 h after the race [10.0 (6.9–15.6) ng/L]. Due to the skewed cTn distribution, mean cTn values were higher at all time-points, and are outlined in Supplementary Table 1. Cardiac TnI values exceeded the 99th percentile (26 ng/L) in 84% of study subjects at 3 h and 18% at 24 h following the race. Cardiac TnT values exceeded the 99th percentile (14 ng/L) in 92% of study subjects at 3 h, and 30% at 24 h following the race. Delta cTnI between 3 h post-race and baseline (ΔcTnI 3-0 h) was 49.9 (29.4–87.0) ng/L. Delta cTnT (ΔcTnT 3-0 h) was 31.7 (20.6–50.1) ng/L. Cardiac troponin values at 24 h after the race were also higher than baseline levels in virtually all subjects;

Table 1
Baseline characteristics of subjects included in the study.

	Total cohort (n = 1002)
Age, years	46.8 (40.1–52.6)
Males, %	782 (78.2%)
BMI, kg/m^2	25.3 (23.7–27.3)
Body weight, kg	82.1 (74.6–89.4)
Systolic blood pressure, mmHg	136 (126–148)
Diastolic blood pressure, mmHg	79 (73–86)
Waist circumference, cm	86.0 (80–92)
Family history of sudden death/early myocardial infarction, n (%)	187 (18.7%)
History of hypertension, n (%)	23 (2.3%)
Current smokers, n (%)	13 (1.3%)
Framingham risk score, %[a]	1 (0–5)
Physical fitness	
Resting heart rate, beats/min	59 (53–67)
MET hours per week[b]	51.3 (31.8–80.0)
Number of races past 5 y, n (%)	7 (3–15)
Self-reported maximal heart rate, beats/min	185 (178–193)
Race performance	
Race duration, hours	3.7 (3.4–4.2)
Maximal heart rate during the race, beats/min	178.0 (170–186)
Maximal heart rate of estimated maximal heart rate, %	100.4 (96.8–104.4)
Mean heart rate during the race, beats/min	157.0 (148.0–165.0)
Mean heart rate of estimated maximal heart rate, %	88.6 (84.5–92.5)
Biomarkers at baseline	
cTnI, ng/L[c]	1.9 (1.6–3.0)
cTnT, ng/L[d]	3.0 (3.0–4.2)
BNP, pg/mL	13.4 (10.0–21.2)
CRP, mg/L	0.7 (0.4–1.3)
Creatinine, umol/L	83.8, SD: 11.7
eGFR, mL/min/1.73m^2	91.3, SD: 12.7
Total Cholesterol, mmol/L	5.1 (4.6–5.8)
LDL, mmol/L	3.2 (2.6–3.7)
HDL, mmol/L	1.5 (1.3–1.7)
Hemoglobin, g/dL	14.5, SD: 1.0

[a] Framingham risk score: 10-year risk of death or myocardial infarction.
[b] MET = Metabolic equivalents of task (3.5 mL O^2/kg/min). Estimated by IPAQ-SF.
[c] 40.2% had cTnI values ≤ 1.6 ng/L (limit of detection).
[d] 58.1% had cTnT values ≤ 3.0 ng/L (limit of blank).

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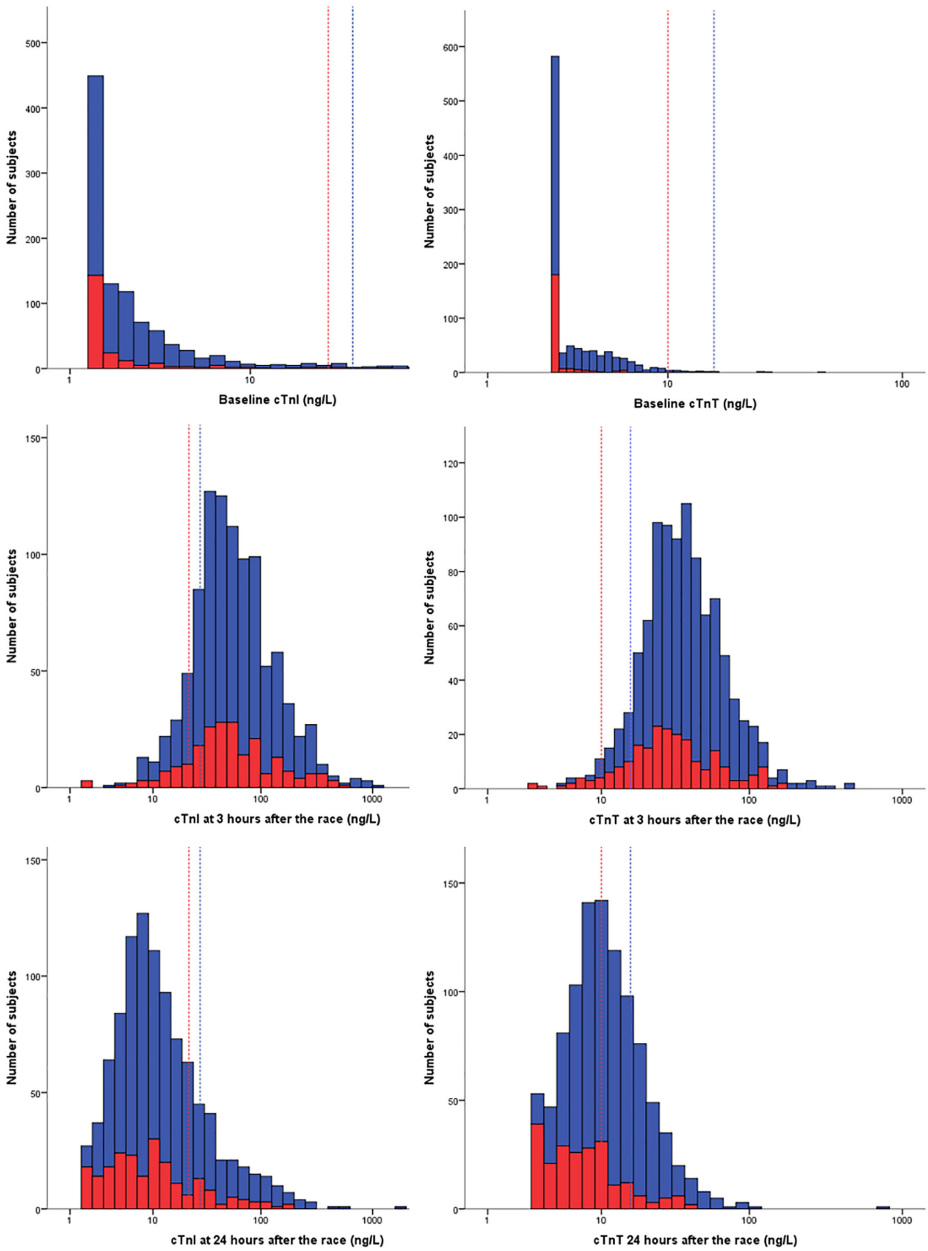

Fig. 1. Distribution of cTn at baseline, and at 3- and 24 h after the race, red indicates female subjects; blue male subjects. Dotted lines represent the sex-specific 99th percentile. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ΔcTnI 24 h–0 h: 7.4 (3.7–16.5) ng/L, ΔcTnT 24 h–0 h: 6.3 (3.3–11.1) ng/L, all $p < 0.001$.

The correlation between cTnI and cTnT at baseline was moderate (rho = 0.60, $p < 0.001$), likely due to a high number of subjects with cTn values below the limit of detection. At 3- and 24 h after the race, the correlation was closer (3 h: rho = 0.87, $p < 0.001$, 24 h: rho = 0.76, $p < 0.001$).

3.2. Factors associated with cTn levels at baseline and following exercise

Baseline systolic blood pressure and race duration were consistently related to both cTnI and cTnT at all time-points following the race both in bivariate and multivariable models (Table 2, Fig. 2). At baseline age, systolic- and diastolic blood pressure, BMI, body weight, waist circumference, Framingham risk score and LDL-levels were positively

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Table 2
Bivariate correlations (Spearman's Rank Correlation) and multiple linear regression models for cTn at different time-points (n = 1002). B signifies the regression coefficient. Variables included in the multiple regression analysis: gender, age, resting heart rate, baseline systolic- and diastolic blood pressure, low density lipoprotein (LDL), estimated glomerular filtration rate (eGFR), body mass index (BMI), race duration, Framingham risk score and baseline cTn values. A backward variable elimination procedure was used.

	cTnI baseline				cTnT at baseline			
	Bivariate correlations		Multiple regression ($R^2 = 0.06$)		Bivariate correlations		Multiple regression ($R^2 = 0.16$)	
	Rho	p-value	B	p-value	Rho	p-value	B	p-value
Age, years	0.17	<0.001		Ns	0.30	<0.001		Ns
Gender, males	0.22	<0.001		Ns	0.26	<0.001	0.110	<0.001
Resting heart rate, bpm	−0.18	<0.001	−0.010	<0.001	−0.14	<0.001	−0.004	0.001
Systolic BP, mmHg	0.21	<0.001	0.005	0.002	0.19	<0.001	0.002	0.004
Race duration, hours	−0.16	<0.001	−0.088	0.008	−0.10	0.003		Ns
eGFR, mL/min/1.73m^2	−0.10	0.001		Ns	−0.23	<0.001	−0.004	<0.001
LDL, mmol/L	0.09	0.005		Ns	0.07	0.03	−0.023	0.084
Framingham risk score, %	0.23	<0.001	0.018	0.004	0.37	<0.001	0.021	<0.001
BMI, kg/m^2	0.10	0.002	0.016	0.035	0.09	0.005		Ns
Body weight, kg	0.18	<0.001			0.17	<0.001		
Waist circumference, cm	0.14	<0.001			0.17	<0.001		
Diastolic BP, mmHg	0.16	<0.001			0.15	<0.001		
MET hours per week	0.05	0.158			0.06	0.059		
HDL, mmol/L	−0.02	0.598			−0.04	0.271		
Endurance training, years	0.12	0.001			0.10	0.005		
Maximal heart rate race, bpm	−0.13	0.004			−0.22	<0.001		
% maximal HR of estimated max	−0.08	0.069			−0.08	0.052		
Mean HR during race, bpm	−0.12	0.006			−0.21	<0.001		
% mean HR of estimated max	−0.07	0.116			−0.07	0.083		
	cTnI 3-h post-race				cTnT 3-h post-race			
	Bivariate correlations		Multiple regression ($R^2 = 0.15$)		Bivariate correlations		Multiple regression ($R^2 = 0.16$)	
	Rho	p-value	B	p-value	Rho	p-value	B	p-value
Age, years	−0.05	0.094	−0.014	0.001	0.03	0.351	−0.004	0.087
Gender, males	0.05	0.152	−0.269	0.002	0.16	<0.001		Ns
Resting heart rate, bpm	−0.03	0.382		Ns	−0.07	0.036		Ns
Systolic BP, mmHg	0.14	<0.001	0.006	<0.001	0.14	<0.001	0.004	<0.001
Race duration, hours	−0.15	<0.001	−0.228	<0.001	−0.25	<0.001	−0.216	<0.001
eGFR, mL/min/1.73m^2	0.03	0.403		Ns	−0.03	0.402		Ns
LDL, mmol/L	−0.02	0.498		Ns	0.09	0.004		Ns
Framingham risk score, %	−0.01	0.73	0.020	0.090	0.09	0.003		Ns
BMI, kg/m^2	0.04	0.225	0.019	0.045	0.02	0.458		Ns
Baseline cTn, ng/L	0.31	<0.001	0.346	<0.001	0.29	<0.001	0.442	<0.001
Body weight, kg	0.08	0.008			0.10	0.002		
Waist circumference, cm	0.03	0.309			0.05	0.131		
Diastolic BP, mmHg	0.06	0.053			0.05	0.137		
MET hours per week	−0.03	0.438			0.03	0.431		
HDL, mmol/L	0.00	0.998			−0.02	0.550		
Endurance training, years	−0.07	0.050			−0.04	0.206		
Maximal heart rate race, bpm	0.10	0.026			0.00	0.917		
% maximal HR of estimated max	0.02	0.606			−0.02	0.62		
Mean HR during race, bpm	0.14	0.001			0.11	0.011		
% mean HR of estimated max	0.07	0.092			0.90	0.048		
	cTnI 24-h post-race				cTnT 24-h post-race			
	Bivariate correlations		Multiple regression ($R^2 = 0.36$)		Bivariate correlations		Multiple regression ($R^2 = 0.28$)	
	Rho	p-value	B	p-value	Rho	p-value	B	p-value
Age, years	0.15	<0.001	0.008	0.008	0.16	<0.001		Ns
Gender, males	0.13	<0.001		Ns	0.30	<0.001	0.092	0.096
Resting heart rate, bpm	−0.08	0.013		Ns	−0.09	0.004		Ns
Systolic BP, mmHg	0.22	<0.001	0.007	<0.001	0.23	<0.001	0.005	<0.001
Race duration, hours	−0.16	<0.001	−0.142	<0.001	−0.28	<0.001	−0.199	<0.001
eGFR, mL/min/1.73m^2	−0.08	0.015		Ns	−0.09	0.006		Ns
LDL, mmol/L	0.07	0.026		Ns	0.09	0.004	0.040	0.067
Framingham risk score, %	0.20	<0.001		Ns	0.37	<0.001		Ns
BMI, kg/m^2	0.09	0.030	0.024	0.010	0.14	<0.001	0.024	<0.001
Baseline cTn, ng/L	0.51	<0.001	0.765	<0.001	0.44	<0.001	0.637	<0.001
Body weight, kg	0.14	<0.001			0.22	<0.001		
Waist circumference, cm	0.11	0.001			0.19	<0.001		
Diastolic BP, mmHg	0.14	<0.001			0.14	<0.001		
MET hours per week	0.02	0.533			0.04	0.182		
HDL, mmol/L	0.00	0.973			−0.09	0.007		
Endurance training, years	0.03	0.415			0.02	0.584		

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Table 2 (*continued*)

	cTnI 24-h post-race				cTnT 24-h post-race			
	Bivariate correlations		Multiple regression ($R^2 = 0.36$)		Bivariate correlations		Multiple regression ($R^2 = 0.28$)	
	Rho	p-value	B	p-value	Rho	p-value	B	p-value
Maximal heart rate race, bpm	−0.03	0.444			−0.05	0.241		
% maximal HR of estimated max	−0.02	0.640			−0.02	0.638		
Mean HR during race, bpm	−0.01	0.850			0.07	0.088		
% mean HR of estimated max	0.02	0.621			0.11	0.009		

correlated with cTn, while resting heart rate, race duration, mean heart rate during the race, and eGFR were negatively correlated (Table 2). A similar pattern was detected for correlations with cTn at 24 h after the race.

Age was inversely associated with the cTnI response at 3 h after the race, but positively correlated at baseline and 24 h after the race.

A total of 219 females (22%) were included into the analysis. Female participants were younger than the male cohort (45.7 (38.8–51.1) versus 47.2 (40.3–53.2) years, p = 0.022). They also had lower systolic blood pressure (127 (120–140) vs 138 (129–150) mmHg, p < 0.001), lower BMI (23.8 (22.2–25.8) vs 25.6 (24.3–27.6) kg/m^2, p < 0.001), and they finished the race 0.8 h slower than their male counterparts (4.5 (4.0–5.0) vs 3.7 (3.3–4.0) hours, p < 0.001).

Both cTnI and cTnT were significantly higher in male as compared with female cyclists both at baseline and at 24 h after the race. At 3 h after the race, male participants had significantly higher cTnT but not cTnI values. The number of participants who exceeded the sex-specific 99th percentile at all time-points was similar for both genders (Supplementary Table 2). Female gender remained a significant predictor for higher cTnI 3 h after the race (B = −0.27, p = 0.002), but not for cTnT. At 24 h after the race, gender was a borderline significant predictor of the cTnT (B = 0.09, p = 0.096), but not the cTnI response.

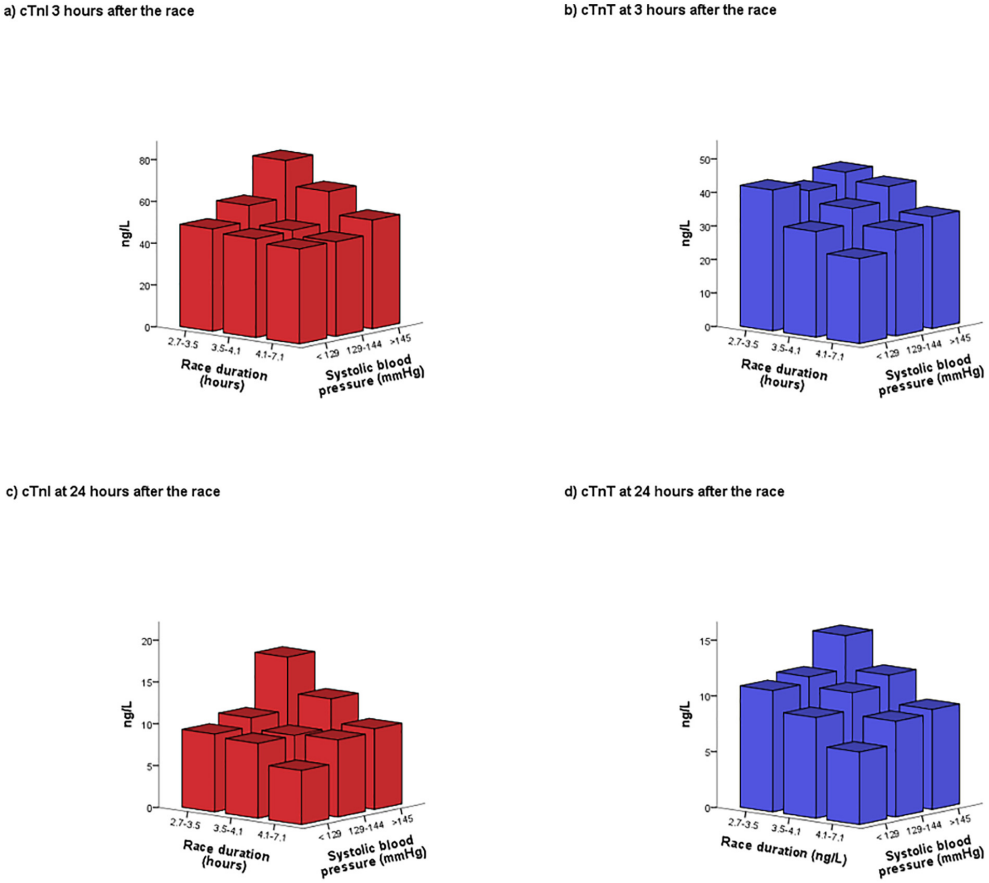

Fig. 2. Median cardiac troponin values plotted against tertiles of race duration and baseline systolic blood pressure a) cTnI 3 h post-race, b) cTnT 3 h post-race, c) cTnI 24 h post-race, d) cTnT 24 h post-race, n = 1002.

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Baseline cTn values were closely associated with the post-exercise cTn values in multiple regression analysis. A secondary analysis on the delta increase in cTn from baseline to 3 h after the race and from baseline to 24 h after the race was performed, and systolic blood pressure and race duration remained independent predictors of the cTn increase in these models (Supplementary Table 3).

Only 55% (n = 551) reported heart rate data from personal sport watches, and heart rate variables were therefore not included in the multiple regression models. An analysis that included the variable "mean heart rate during the race" was performed in the cohort with heart rate data. In this model, the associations between mean heart rate during the race and cTnI at 3-h post-race (B = 0.004, p = 0.077) and with cTnT 24 h post-race (B = 0.003, p = 0.028) were borderline significant. Mean heart rate did not remain a significant predictor for cTnT 3 h post-race or cTnI 24 h after the race.

Levels of low-density lipoprotein cholesterol (LDL-C) at baseline were not associated with the cTn response at either 3 or 24 h after the race, nor were the Framingham risk score (Table 2). A total of 31 subjects reported a first degree relative with premature cardiovascular disease (<50 years of age). These subjects did not have a different cTn response than the rest of the cohort. Subjects above the age of 35 years that fulfilled the High-Risk criteria proposed for recreational athletes (n = 238, 23.8%) were also assessed separately. Increased BMI was the most common cause for High-Risk classification (n = 183, 76.9%, Supplementary Fig. 3). High-Risk individuals had similar levels of cTn at 3 h after the race and higher cTn levels at 24 h post-race (cTnI: 12.3 (6.8–25.6) vs 9.6 (5.9–18.6) ng/L, p = 0.001, cTnT: 11.5 (7.8–18.5) vs 9.9 (6.7–15.0) ng/L, p < 0.001, Supplementary Fig. 4).

Some of the included subjects reported several co-morbidities and intake of different supplements. (Supplementary Table 4–5): 31.8% of subjects (n = 319) reported to use supplements regularly, while 27.7% of subjects (n = 278) never used supplements. There were no difference in cTn kinetics between subjects who used supplements and those who did not.

4. Discussion

This is the largest study ever performed to determine the predictors of the exercise-induced cTn response. Troponin levels increased in all subjects, with >84% of subjects exceeding the 99th percentile of the cTn assays at 3 h following exercise. At 24 h 18–30% of subjects still had cTn levels above the 99th percentile. Systolic blood pressure and race duration were consistent predictors of cTnT and cTnI levels at all time-points following exercise. Other previously suggested variables were less consistently associated with the cTn increase. The strength of this study is the large number of study subjects, allowing the inclusion of all previously suggested predictors of the exercise-induced cTn increase, the use of two different high-sensitivity cTn assays, and multiple cTn sample time-points. However, despite the large sample size allowing inclusion of all previously suggested predictors, the post-race multiple regression models only explained a variance of 15–36% of cTn levels following exercise. Considering this, the present study shows that additional unidentified factors are involved in and needed to improve the prediction and understanding of the exercise-induced cTn response.

4.1. Exercise-induced cTn elevation

There is limited understanding of the underlying mechanisms of the physiological cTn increase following exercise. A leading current hypothesis is that stress can cause reversible cardiac injury leading to cell wounds, cytoplasmatic blebbing, and the release of intracellular macromolecules, as well as activation of apoptosis [6]. Irreversible injury with necrosis of cardiomyocytes and degradation of cTn by lysosomal enzymes are however, difficult to distinguish from the reversible causes based on systemic cTn levels alone, and current imaging modalities lack the sensitivity to identify non-focal necrosis [6]. Several mechanisms causing exercise-induced cTn release have been proposed. It has been hypothesized that cTn can be released due to exercise-induced increase in wall tension and ventricular strain, neuro-hormonal stimulation and/or reversible ischemia due to increased myocardial energy demand [2,6,7].

The present study demonstrates an increase in both cTnI and cTnT in all subjects following strenuous exercise. This finding supports that exercise-induced cTn increase in healthy subjects is a physiological response.

4.2. Exercise intensity and duration

In this study, there was a consistent inverse relation between cTn levels and race duration in both bivariate and multiple regression models (Table 2, Supplementary Table 3). Previous studies assessing the relationship between the cTn response and race duration have been conflicting: Some studies found a direct correlation [3,16,17], whereas others found an inverse correlation [4,13] or no correlation [11,12,18]. Although race duration is a readily available parameter, the interpretation of this variable is complex. Race duration is related to physical fitness [19]. However, it also reflects sport specific technical skills, exercise intensity and the duration of high-intensity work. Shorter race duration requires higher velocity, which necessitates higher exercise intensity. Exercise intensity and the duration of high-intensity work are important predictors of the exercise-induced cTn response. The intensity of the work required to induce a significant increase in the exercise-induced cTn response has recently been addressed by Stewart et al. [20]. In their study, a marked increase in exercise-induced cTn was found following a 90 min ergometer cycling test, when exercise was performed with an exercise intensity above the gas exchange threshold. The present population-based study and the mechanistic study by Stewart et al. underscore the importance of the intensity-duration domain as an important determinant for cTn elevation.

4.3. Systolic blood pressure

A major finding of the present study was the consistent relationship between systolic blood pressure measured prior to the race and cTn elevations both at 3- and 24 h after the race. Systolic blood pressure has not been included in the multiple regression analyses in previous studies [10,12,13]. However, some smaller studies have reported bivariate correlations between blood pressure and exercise-induced cTn increase [21,22]. Our finding is intriguing, and in line with the recent mechanistic study by Weil and al., that observed a transient increase in cTnI following phenylepinephrine infusion in a pig model [7]. The phenylepinephrine infusion caused increased systolic blood pressure and increased left ventricular end diastolic pressure in the absence of ischaemia. Our findings and the mechanistic work by Weil et al., suggest that the exercise-induced cTn increase in healthy subjects is related to increase in cardiac work, both in response to mechanical work and potentially due to increased neuro-hormonal activity induced by strenuous physical exercise and the competitive situation.

4.4. Body composition

The present study found inconsistent correlations between cTn and BMI, body weight and waist circumference at 3- and 24 h after the race. There are conflicting reports on the relationship between cTn and BMI underscoring the complexity of this association; Eijsvogels et al. found no significant association with exercise-induced cTn increase [9], while a meta-regression analysis found increased body weight to be a major predictor [4]. BMI was originally established to measure tissue mass and obesity [23]. However, BMI does not reflect body tissue composition. In a healthy athletic population, increased

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weight may reflect a higher muscular proportion compared with a larger proportion of adipose tissue in a sedentary population [24]. Increased BMI may increase work load during physical exercise and thereby influence the levels of work-load dependent biomarkers. The interpretation of the relationship between BMI and exercise-induced cTn response, however requires careful interpretations, particularly in relation to a potential collinearity between body weight and performance [4]. Waist circumference was also used to assess the potential impact of body composition on the exercise-induced cTn response. Waist circumference did not provide additional benefit compared with BMI in the prediction of the exercise-induced cTn increase.

4.5. Age and gender

Following exercise there was no clear relationship between age and cTn levels. These inconsistent results are in line with previous studies that present conflicting data on the relationship between age and the exercise-induced cTn release: some studies indicate increased cTn levels in younger subjects [11–13], some studies indicate increased cTn levels in older athletes [10,25], whereas others report no correlations [26,27]. Our study suggests that age is not a major independent predictor of exercise-induced cTn increase. However, since only 61 subjects (6.1%) were above 60 years of age, future studies will need to confirm our findings in subjects above middle-age.

Women have lower cTn levels at baseline than men, and gender-specific cTn cut-off values have been proposed. Gender differences have also been found to influence the exercise-induced cTn release in some studies [11,28]. In our cohort, the number of subjects who exceeded the gender-specific cTn cut-off at all time-points was similar for male and female participants, and the cTn distributions were fairly equal (Fig. 1, Supplementary Table 2). In multiple regression analysis, however, females were found to have a higher cTnI increase as compared to men at 3 h after the race when adjusted for other variables. This finding was not identified for the cTnT assay.

4.6. Training experience

Training experience has been found to be inversely associated with post-exercise cTn in several studies [8,10,11]. The present study used several measures to estimate training condition. Training and competitive experience was measured as number of years of endurance training and number of endurance exercise competitions during the past five years. No significant association was found between this measure and cTn levels. The International Physical Activity Questionnaire (IPAQ) was used to assess the amount of exercise prior to the race. No relation was found between this measure and exercise-induced cTn. Our findings argue against a major relationship between training experience and cTn response.

4.7. Other cardiovascular risk factors

CV risk factors like cholesterol levels, family history of premature CV disease and Framingham risk were not found to significantly affect the exercise-induced cTn increase.

The ESC sports cardiology group has proposed specific criteria for identifying recreational athletes above 35 years of age at increased risk of sport-related cardiac events [29]. Using the proposed criteria, a total of 238 (23.8%) of our participants were classified as High-Risk individuals due to the presence of at least one CV risk factor. A higher cTn level 24 h after the race was identified in this High-risk group ($p < 0.01$, Supplementary Fig. 4). The clinical implications of this finding remain to be determined.

4.8. Limitations

There are some limitations that apply to the current study: First, with this large sample size, it was impossible to include mechanistic data beyond biomarkers and biometrics acquired during the study. The major limitations therefore relate to the lack of mechanistic data such as echocardiographic and ischemia assessment. Second, the present cohort was primarily middle-aged male subjects, and the findings may therefore not apply in a very young (<20 years of age) or an above middle aged (>60 year of age) population. Third, undiagnosed coronary artery disease may be prevalent in this population. The impact of coronary artery disease on exercise-induced cTn increase remains to be elucidated. Fourth, the clinical implications of the current findings need to be determined. The clinical implications will be assessed by pre-specified follow-up studies at 5-, 10- and 20 years following inclusion. Fifth, the present cohort consisted of recreational athletes with a higher fitness level compared with the general population. The impact of cardiovascular adaptations to long-term physical activity, i.e. athlete's heart, on exercise-induced cTn increase was not assessed in the present study. Sixth, the number of female subjects included in this study is much lower than the number of male subjects. This should be considered when interpreting the results on sex-specific cTn results. Seventh, heart rate data was based upon self-reported sport watch measurements from study subjects. Data variability between different brands was not considered.

5. Conclusion

In this large-scale prospective observational study, systolic blood pressure and race duration were consistent predictors of the exercise-induced cTn increase. These variables were more important than previously reported predictors of the exercise-induced cTn increase, such as body mass index, age, gender or training experience.

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Conflict of interest

ØK, MB, TM, TA, BA, OJG and SØ have no conflicts of interest to declare. Modest conflicts of interest have been reported by ØS, KMA, RB and TO. ØS has received lecture fees from Abbott Diagnostics. KMA has served on one advisory board for Roche Diagnostics. RB is a board member of the North Sea Race organization. TO has served on advisory boards for Abbott Diagnostics and Roche Diagnostics, and has received research support from Abbott Diagnostics and Roche Diagnostics via Akershus University Hospital, and speaker's honoraria from Roche Diagnostics.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2019.02.044.

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IV

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ORIGINAL ARTICLE

WILEY

High physical fitness is associated with reduction in basal- and exercise-induced inflammation

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C-reactive protein (CRP) increases after strenuous exercise. It has been a concern that prolonged strenuous exercise may be harmful and induce a deleterious inflammatory response. The purpose of this study was to (a) assess and quantify the magnitude of CRP response following an endurance cycling competition in healthy middle-aged recreational cyclists. (b) Identify important determinants of this response. (c) Identify the relationship between CRP, myocardial damage (cardiac Troponin I (cTnI)), and myocardial strain (B-type natriuretic peptide [BNP]). (d) Identify the relationship between CRP and clinical events, defined as utilization of healthcare services or self-reported unusual discomfort. Race time was used as a measure of physical fitness. A total of 97 individuals (43±10 years of age, 74 [76%] males) were assessed prior to and 0, 3, and 24 hours following the 91-km mountain bike race "Nordsjørittet" (Sandnes, Norway, June 2013). There was a highly significant increase in CRP from baseline to 24 hours (0.9 (0.5-1.8) mg/L vs. 11.6 (6.0-17.5) mg/L (median[IQR]), $P<.001$), with no correlation of CRP to cTnI and BNP at any time-point. CRP was strongly correlated to race time at baseline ($r=.38$, $P<.001$) and at 24 hours following the race ($r=.43$, $P<.001$), In multivariate models, race time was an independent predictor of CRP both at baseline and at 24 hours ($P<.01$). There was no relationship between CRP levels and clinical events. In conclusion, high physical fitness was associated with reduction in both basal- and exercise-induced CRP. No adverse relationship was found between high intensity physical exercise, CRP levels, and outcomes.

KEYWORDS
bicycling, cardiovascular risk factors, C-reactive protein, endurance exercise, healthy individuals, recreational sport

1 | INTRODUCTION

Resting levels of the inflammatory marker C-reactive protein (CRP) are strongly associated with increased risk of cardiovascular (CV) events.[1-4] An inverse relationship between physical fitness and basal CRP has been demonstrated.[5,6] Increased physical activity is associated with reduced risk of CV events.[7-10] Physical activity is therefore recommended for the prevention of CV disease.[11] Participation in recreational sports competitions represents an important motivation

to many that perform physical training on a regular basis. However, there is a concern that the strenuous physical activity during these competitive events may be harmful.[12-15] Previous studies have suggested a small but significant increased risk of sudden death related to strenuous physical exercise.[16-19] Following physical exercise, there is an increase in CRP levels.[7,20-22] This increase in CRP has not been associated with adverse events in prior studies that have included highly selected populations of well-trained athletes participating in ultra-endurance events and marathons.[20,22,23] There are limited data on the relationship between prolonged high intensity physical exercise and the inflammatory response in unselected populations of recreational sport participants with a larger range of fitness. Medium length recreational sport bicycle races are less physically demanding than marathons. It may therefore be anticipated that these bike races attract a population of participants with a greater range of physical fitness at greater risk of CV disease.

The aim of this study was to (a) assess and quantify the magnitude of the exercise-induced CRP response following an endurance cycling competition in presumably healthy middle-aged recreational cyclists. (b) Identify important determinants of the CRP response following exercise, and in particular to assess the influence of physical fitness on baseline and post-exercise CRP levels. (c) Identify the relationship between CRP levels and markers of myocardial damage (cardiac Troponin I [cTnI] and myocardial strain (B-type natriuretic peptide [BNP]). (d) Identify the relationship between CRP levels and clinical events for the first week following the race. Clinical events were defined as either utilization of healthcare services or self-reported unusual discomfort.

2 | METHODS

This is a prospective observational study. Study participants were recruited among cyclists participating in the 2013 edition of the 91-km North Sea Race ("Nordsjørittet").[21] This is an annual mountain bike race attracting approximately 12 500 participants with a great range of different fitness levels. The regional ethics committee (2013/550/REK vest) approved the study. All participants gave written informed consent prior to enrollment into the study.

2.1 | Study population

Participants in the North Sea Race 2013 without medical treatment and any known disease were invited to participate in the study. All study participants were recruited by an electronic form, distributed on the Internet through the official website of the North Sea Race (www.nordsjorittet.no). Eligible participants that signed the informed consent and completed the four blood samples were included in the study.

2.2 | Acquisition of blood samples, patient demographics, and biometric measurements

Blood samples, resting ECG, blood pressure, and bodyweight were acquired approximately 24 hours prior to the race (baseline) and 0, 3, and 24 hours following the race. Detailed information regarding demographics, medical history, degree of fitness, physical condition, and nutrition was acquired by six electronic questionnaires (Adobe FormsCentral, Adobe Systems Software Ireland Ltd., Ireland). All participants were advised to use a heart rate monitor to determine the individual heart rate during the competition. Heart rate was reported both as median and max heart rate. Heart rate was also transformed into percent of max age-adjusted heart rage: HRmax=208-0.7* age (years).[24] Race time was recorded by an electronic timekeeping system supplied by the organizer of the race.

Venous blood samples were acquired at all the four timepoints. For hematology and HbA1c, 3-mL K2 EDTA tubes were used. For CRP, creatine kinase (CK), creatinine, and estimated glomerular filtration rate (eGFR) serum analyses were used. For CRP, a high sensitivity assay was used. Serum separation tubes with K2 ethylenediaminetetraacetic acid (EDTA) were used for analyses of BNP. The troponins were analyzed using a high-sensitive cardiac troponin I STAT assay from Abbott Diagnostics with a lower limit of detection of 1.6 ng/L. The tubes were stored in a refrigerator until analysis. Tubes without any additives were placed in room temperature and centrifuged after coagulation at 2000 *g* for 10 minutes. Following centrifugation, all blood samples were stored at +4°C and transported to Stavanger University Hospital for analysis within 24 hours. The biomarkers were analyzed using Architect c16000TM and Abbott Architect i2000TM (Abbott Diagnostics, IL, USA) (clinical chemistry) and Variant Turbo II (Bio-Rad Laboratories, Hercules, CA, USA) (HbA1c).

2.3 | Determination of physical fitness

Physical fitness has been defined as "The ability to carry out daily tasks with vigor and alertness, without undue fatigue and with ample energy to enjoy (leisure) pursuits and to meet unforeseen emergencies".[25] Physical fitness is operationalized as "(a set of) measurable health and skill-related attributes" that include cardiorespiratory fitness, muscular strength and endurance, body composition and flexibility, balance, agility, reaction time and power.[11] Race time in a bike race reflects all the core attributes of physical fitness: cardiorespiratory fitness, muscular strength and endurance, body composition and flexibility, balance, agility, reaction time, and power. Race time was therefore chosen as the primary measure for physical fitness.

During a competitive event, accidents or other obstacles that preclude the interpretation of race time as marker of physical fitness may occur. We therefore also included a self-evaluation

of physical fitness that the participants had to hand in prior to the race. In this self-evaluation of physical fitness, the study participants had to grade their own fitness level (5 levels) compared with other contestants at the same age. In addition, we also attempted to assess prior history of physical fitness by the number of participations in recreational sport competitions during the past 5 years, and their training level by the number of training hours per week during the past 3 months prior to the race.

2.4 | Clinical outcomes

All study participants reported clinical events 1 week after the race. Clinical endpoints were defined as any unscheduled healthcare contact, sick leave, or any kind of unusual discomfort following the race. Discomfort was defined as an affirmative response to the following question 1 week following the race: "Have you been experiencing more than expected pain or exhaustion following the race?"

2.5 | Statistics

In accordance with a previous study in marathon runners, the aim was to include 100 study subjects.[20] With 100 patients, bivariate correlations of size $|r|=.27$ have a power of 80%, and for $|r|=.32$, there is a power of 90%, both at a two-sided 0.05 significance level. Mean and standard deviation (SD) were used to report continuous variables, and median and interquartile range (IQR) were used to report markedly skewed distributions. Nonparametric tests were used for the assessment of correlations (Spearman) and longitudinal changes (Mann-Whitney *U* test) and comparisons between and within groups (Kruskal-Wallis and Wilcoxon). Regression analyses are justified based on asymptotic normality due to the fairly large size of the data set. Backward stepwise variable selection was used in the regression analyses. The covariates that were included in the model were variables known to influence CRP: baseline CV risk factors, physical fitness, and muscle damage (only following exercise for CRP). A two-tailed *P*-value of <.05 was considered significant. For all statistical analyses, the statistical package SPSS version 23 (IBM, NY, USA) and R (R Foundation for Statistical Computing, Vienna, Austria) were used.

3 | RESULTS

Ninety-seven healthy cyclists were included in the study (Table 1). Study participants were 42.8±9.6 years of age, 74 (76%) were males, 43 (44%) had never smoked. There were no diabetics. Mean race time was 4:22±0:52 (hour:minute) with a mean heart rate of 156±17 bpm which is 86±14% of estimated age-adjusted max heart rate. There was a large range in training hours per week among study subjects; one study participant logged 28 training hours/week, and six logged <3 hours/week. Likewise, there was a large range in competitive activity during last 5 years prior to the study: 10 subjects had no competitive experience, whereas 13 subjects had participated in more than 30 competitions.

TABLE 1 Baseline characteristics and competition performance of the study participants, North Sea Race, Sandnes, Norway, June 2013 (n=97), mean±SD or median (IQR) for markedly skewed distributions, or number, %

Gender (male, %)	74 (76.3)
Age (y)	42.8±9.6
Weight (kg) Body mass index (kg/m^2)	83.4±14.0 25.3 (23.4-28)
Blood pressure (mm Hg)	
Systolic	138 (129-152)
Diastolic	77 (71-85)
Family history of CV disease (n, %)	25 (26)
Smoker (n, %)	
Never	49(51)
Stopped	39(40)
Current	4(4)
Baseline blood tests	
HbA1c (%)	5.3±0.27
Total cholesterol (mmol/L)	4.9 (4.4-5.5)
HDL (mmol/L)	1.3 (1.1-1.6)
LDL (mmol/L)	3.2±0.84
eGFR (ml/min/1,73 m^2)	94±13.9
ECG: left ventricular hypertrophy (n, %)	29 (30)
Training history	
No. of endurance competitions past 5 y	7 (2-16)
Hours of training per week past 3 mo	7.0 (5-10)
North Sea Race 2013	
Finishing time (h:min)	4.1 (3.6-4.7)
Max heart rate (bpm)	180 (174-188)
Max heart rate of expected max heart rate (%)	96.7 (95-100)
Mean heart rate (bpm)	158 (148-168)
Mean heart rate of expected max (%)	86 (82-88)

3.1 | CRP levels before and after exercise

There was a highly significant increase in CRP from baseline to 24 hours (0.9 [0.5-1.8] mg/L vs. 11.6 [6.0-17.5] mg/L, *P*<.001) (median [IQR]). CRP values at baseline and at 24 hours following the race were not correlated to gender, age, or injury during the race (Table 2).

3.2 | Physical fitness and CRP levels

There was a highly significant (*P*<.001) increase in CRP following exercise in all four measures of physical fitness, with the highest CRP levels in the least fit study participants (Figure 1).

TABLE 2 Baseline C-reactive protein (CRP) and CRP 24 h after the race, *P* values within subgroups, North Sea Race, Sandnes, Norway, June 2013

	CRP (Baseline) median (IQR)	*P*-value	CRP (24 h) median (IQR)	*P*-value
Sex				
Women (24%)	0.8 (0.5-2.0)		11.0 (4.7-21.2)	
Men (76%)	1.0 (0.5-1.8)	.71	11.7 (6.2-17.4)	.96
Age (y of age)				
>42 (55%)	1.0 (0.5-2.0)		8.6 (4.2-17.5)	
<42 (45%)	0.8 (0.5-1.9)	.75	11.8 (8.0-17.7)	.10
Smoking status				
Never (51%)	0.8 (0.5-1.5)		9.8 (5.8-15.7)	
Previous (40%)	1.0 (0.5-2.1)		12.9 (5.1-20.6)	
Current smoker (4%)	1.3 (0.8-6.8)	.23	16.2 (10.3-35.5)	.21
BMI (kg/m^2)				
20-25 (47%)	0.6 (0.4-1.4)		9.6 (4.2-17.5)	
25-30 (38%)	1.2 (0.5-1.6)		10.7 (7.2-15.5)	
30+(14%)	2.2 (1.0-4.6)	.003	19.5 (11.0-27.9)	.03
Systolic blood pressure (mm Hg)				
<120 (4.1%)	0.8 (0.5-3.3)		14.9 (7.2-20.7)	
120-140 (53.1%)	0.9 (0.5-1.6)		11.5 (6.4-17.5)	
>140 (41.8%)	1.1 (0.5-2.0)	.81	11.5 (4.3-17.8)	.52
Training last 5 days prior to the race				
0-1 h (57%)	1.2 (0.5-2.1)		11.8 (6.8-19.0)	
2-4 h (35%)	0.8 (0.4-1.5)		12.8 (7.2-17.5)	
More than 5 h (8%)	0.6 (0.4-0.8)	.11	3.8 (2.0-7.0)	.003
Injury during race				
Yes (9%)	0.6 (0.4-2.4)		8.1 (3.0-22.3)	
No (86%)	0.9 (0.5-1.8)	.91	11.7 (6.0-17.4)	.91
Medical assistance after the race				
Yes (3%)	1.4 (0.9-1.4)		14.2 (10.3-14.2)	
No (97%)	0.8 (0.5-1.8)	.42	11.6 (5.6-17.5)	.44
Unusual discomfort after the race				
Yes (9%)	1.0 (0.4-1.7)		14.2 (4.0-21.7)	
No (91%)	0.8 (0.5-1.9)	.81	11.6 (6.0-17.4)	.76

Study participants with the fastest race times (Q1) were taller and consisted only of men; they also reported higher self-assessed fitness level (Table 3). There were no differences in age and BMI between riders in the different race time quartiles (Table 3).

Figure 1 illustrates the differences in CRP levels between different measures of physical fitness and training. When participants were divided according to race time quartile (Figure 1A), there was a significant difference between CRP levels at baseline ($P=.004$), immediately after the race ($P<.001$), 3 hours after the race ($P<.001$), and 24 hour after the race ($P<.001$). When patients were divided into quartiles based on number of previous competitions (Figure 1C), there was no significant difference in CRP levels at baseline ($P=.152$) but highly significant differences following competition: immediately after the race ($P<.001$), 3 hours after the race ($P=.009$), and 24 hour after the race ($P<.001$). When patients were asked to assess personal degree of fitness (Figure 1B), the majority of participants answered "good" or "moderate" (n=67). There was a significant difference between groups at baseline ($P=.01$), immediately after the race ($P=.001$), 3 hours after the race ($P<.001$), and 24 hour after the race ($P<.001$). When patients were divided into quartiles of training hours per week (Figure 1D), there was no significant difference until 24 hours after the competition: baseline ($P=.20$), immediately after the race ($P=.061$), 3 hours after the race ($P=.04$), and 24 hour after the race ($P<.016$).

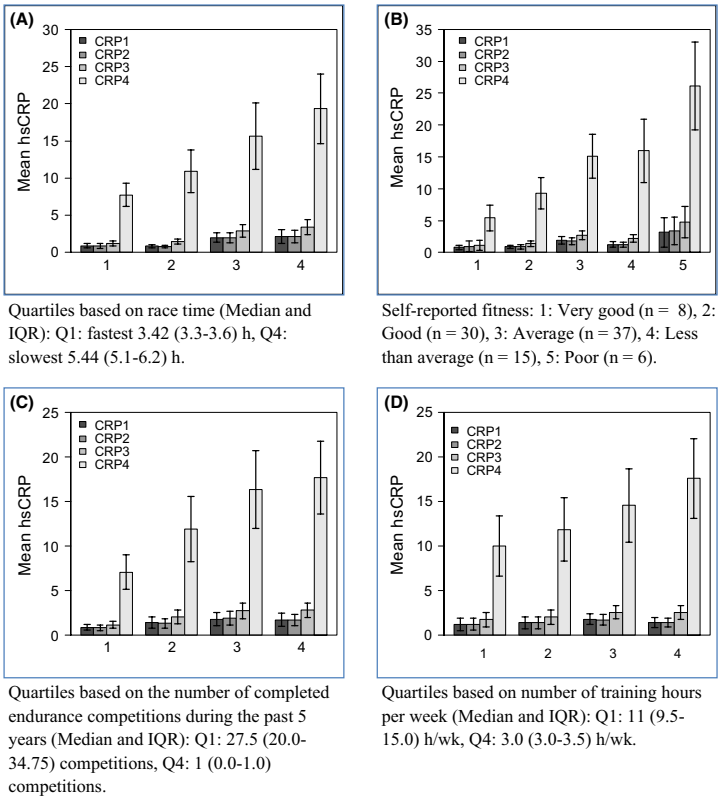

FIGURE 1 The figure illustrates the relationship between CRP levels before and after the race in relation to different measures of physical fitness and training. The bars represent mean (SD) CRP values before and after the race. CRP is assessed 24 h prior to the race (CRP1), immediately following the race (CRP2), 3 h following the race (CRP3), and at 24 h following the race (CRP4). A: The relationship between CRP and race time divided into quartiles with Q1 being the fastest and Q4 being the slowest. B: self-reported degree of fitness. C: number of completed endurance competitions during the last 5 y. D: quartiles based upon the number of reported training hours per week during the last 3 months prior to the race. There was a highly significant ($P<.0001$) increase between CRP1 and CRP4 in all groups. North Sea Race, Sandnes, Norway, June 2013

Quartiles based on race time (Median and IQR): Q1: fastest 3.42 (3.3-3.6) h, Q4: slowest 5.44 (5.1-6.2) h.

Self-reported fitness: 1: Very good (n = 8), 2: Good (n = 30), 3: Average (n = 37), 4: Less than average (n = 15), 5: Poor (n = 6).

Quartiles based on the number of completed endurance competitions during the past 5 years (Median and IQR): Q1: 27.5 (20.0-34.75) competitions, Q4: 1 (0.0-1.0) competitions.

Quartiles based on number of training hours per week (Median and IQR): Q1: 11 (9.5-15.0) h/wk, Q4: 3.0 (3.0-3.5) h/wk.

3.3 | Predictors of CRP levels before and after exercise

Physical fitness assessed as race time was significantly correlated with baseline and 24-hour CRP levels both in uni- and multiple regression analysis. In contrast, among the other measures of physical fitness, only self-perceived physical fitness level was a significant predictor of CRP levels 24 hours following exercise in multiple regression models. At baseline, BMI was a significant predictor of CRP levels both in univariate and in multiple regression models (Table 4). BMI was not a predictor of CRP following physical exercise.

There was a 2.0±6.3% reduction in plasma volume following the race. Plasma volume was calculated by the formula proposed by Dill and Costill.[26] Plasma volume was normalized 3 hour following the race. There was no correlation between plasma volume, CRP, or race time at any time-point.

3.4 | CRP levels and markers of myocardial damage, myocardial strain, and clinical events

There was a highly significant ($P<.001$) increase in cTnI (3.3 [2.0-4.8]-12.7 [4.7-24.5]) and BNP (13.7[10.0-22.2]-34.9[21.0-51.3]) immediately following physical exercise. However, there was no significant relationship between CRP and any of these markers at any time-point before or after the race. For the first week following the race, three participants (3.1%) sought medical advice due to minor infections or symptoms of discomfort. Nine participants (9.3%) reported unusual discomfort without seeking medical attention. There was no difference in CRP levels at baseline or 24 hours following the race between these individuals and the rest of the participants.

4 | DISCUSSION

This is the first study to demonstrate that high physical fitness attenuates CRP increase following strenuous cycling. We found that high physical fitness was associated with attenuated basal inflammation in spite of higher training loads prior to CRP measurements in the most fit study participants. There was no relationship between CRP and markers of myocardial damage (cTnI), myocardial strain (BNP), or clinical events following strenuous physical exercise. However, following physical exercise, there was a correlation between CRP and CK indicating a link between muscular strain and inflammation. In

TABLE 3 Study participants were categorized according to fitness levels based upon their race time quartiles, fastest (Q1) to slowest (Q4), mean±SD, median (IQR) if markedly skewed distribution, or number, %

	Race time (h)				
	1. Quartile (3.4[3.3-3.6])	**2. Quartile (3.9[2.9-4.1])**	**3. Quartile (4.5[4.2-4.7])**	**4. Quartile (5.4[5.1-6.2])**	***P* value**
Baseline characteristics					
Age (y)	40.3±9.8	44.7±11.4	44.3±7.6	41.8±9.4	.605
Height (cm)	183.0±6.7	179.6±7.0	175.5±7.2	175.2±8.9	.001
Female (n, %)	0(0)	5(20)	6(25)	12(50)	.006
Median heart rate during race (bpm)	161(151-172)	160(151-165)	155(144-169)	152(144-163)	.088
Weight (kg)	83.8±8.7	82.3±12.9	80.4±14.3	85.7±17.3	.874
BMI (kg/m^2)	25.0±2.0	25.4±3.2	25.9±3.4	27.9±5.1	.100
Training status (median (IQR))					
Years of training	5.0(3.8-11.3)	8.0(5.0-1.7)	6.5(3.0-15.0)	2.5(1.0-16.3)	.030
No of competitions/5 y	17.0(10.8-32.8)	11.0(5.0-19.0)	3.5(1.0-7.3)	1.0(0.3-4.3)	<.001
Training hours/week past 3 mo	7.5(5.0-9.9)	6.0(3.5-9.0)	5.25(4.3-9.6)	5.0(3.0-7.0)	.106
Self-reported fitness level	1.0(1.0-1.3)	1.0(1.0-2.0)	2.0(1.8-2.0)	3.0 (2.0-3.0)	<.001
Biomarkers					
CRP baseline (mg/L)	0.5(0.4-1.3)	0.6(0.4-1.4)	1.6(0.8-3.8)	1.3(0.5-2.3)	.004
CRP 24 h after race (mg/L)	7.3(4.5-9.6)	11.8(4.1-15.7)	12.8(7.7-21.2)	18.5(9.3-25.0)	<.001
HbA1c (%)	5.3±0.3	5.2±0.2	5.3±0.3	5.2±0.3	.550
Total cholesterol (mmol/L)	4.6±0.6	5.0±0.8	5.3±1.1	4.9±0.7	.077
HDL (mmol/L)	1.5(1.3-1.5)	1.3(1.1-1.6)	1.3(1.0-1.5)	1.2(1.1-1.7)	.155
eGFR (ml/min/1.73 m^2)	95.8±13.6	94.1±15.1	92.2±13.7	95.9±13.7	.758

Self-reported fitness levels: 0: best, 4: worst. North Sea Race, Sandnes, Norway, June 2013.

TABLE 4 Multiple regression analysis (backward) at baseline and 24 h after the race using the following covariables: Age, gender, smoking status, HbA1c, creatine kinase (CK), body mass index (BMI), race time, number of endurance competitions over the last 5 y, number of training hours last 3 mo, and self-reported fitness level. North Sea Race, Sandnes, Norway, June 2013

	Unstandardized coefficients		Standardized coefficients		
	B	**SE**	**Beta**	***t***	***P* value**
CRP baseline (R^2=.23)					
BMI	0.13	0.041	0.32	3.33	.001
Race time	0.49	0.179	0.26	2.72	.008
CRP 24 h (R^2=.31)					
Race time	3.28	1.25	0.29	2.63	.01
Self-reported fitness	2.76	1.07	0.28	2.57	.012
CK (3 h)	0.006	0.002	0.24	2.68	.009

contrast to previous publications, all relationships were independent of age and gender. Our findings may lead us to speculate that the beneficial effects of high physical fitness may not only relate to a favorable modulation of basal inflammation but also an improved response to physical stress (ie, attenuation of potential inappropriate inflammatory responses). This hypothesis has to be further investigated in a larger trial.

Our findings are supported by a recent study in 45 marathon runners[27] that found a correlation between race time, cartilage markers, and exercise-induced inflammation. Our study adds to this study by several findings. The strong correlation between race time and baseline CRP suggests that race time reflects an attribute that exists prior to the competition that modulates the CRP response. We believe that race time in this context is a surrogate for physical fitness, underscoring the important role for physical fitness in the modulation of the inflammatory response. Whereas the interaction between BMI and CRP in patients with established CVD may

be rather complex,[28] the additional effects of BMI on the prediction of baseline CRP in the multivariable models suggest an additional impact of body weight, potentially as a marker of the long-term biological response to physical exercise.

Following physical exercise, a strong correlation between CRP levels and the muscle enzyme CK was found both in uni- and multivariable models. CK is a marker of muscular strain or damage that increases with muscular overload and eccentric work.[29] Within skeletal muscle of healthy individuals, cytokines such as interleukin-6 (IL-6) are released following physical exercise, often referred to as myokines, inducing adaptive responses within the muscles.[30-32] IL-6 is a major stimulant of CRP production in the liver, and it is possible that the increase in CRP observed in the present study involved release of this myokine.[30,33] The magnitude of the CRP response appears to be less prominent in the present study compared with previous studies in marathon runners; only a ninefold in our study as compared with a 21-fold increase in CRP in marathon runners.[20,22] Interestingly, in these marathon runners, there was a markedly lower baseline CRP compared with our study of cyclists. CRP assays, blood sampling, and different study populations may account for some of the difference. However, cycling is almost entirely a concentric muscular activity as compared to running that has a larger proportion of eccentric muscular work.[34] In concentric muscular activity, there are less microruptures of the muscle fibers than in eccentric muscular activity and therefore less inflammation.[34-36]

The present study underscores the potential beneficial effects of physical exercise on the CRP response both before and following physical exercise. All measures of physical fitness, training level, or competitive experience suggest a beneficial effect of increased physical activity on the inflammatory response. However, only measures such as race time, BMI, and CK remained predictors of the CRP response in multiple regression models. These findings suggest that it may be necessary to increase physical performance in order to improve the CRP response to stress. This finding has a potentially very important implication: If a training intervention does not improve physical performance, improvement in the inflammatory response cannot be anticipated.

Elevated basal CRP levels are associated whit an increased risk of CV events in epidemiological studies.[37,38] It is a concern that excessive physical activity may precipitate an adverse inflammatory response. In the present study, however, there was no evidence of a short-term deleterious effect of exercise-induced inflammation as suggested by a lack of a relationship between CRP and markers of myocardial damage, myocardial strain, or clinical events the first week following physical exercise.

4.1 | Limitations

The inflammatory response in the present study was modest, and the number of clinical events was small. Our assessment of the relationship between CRP and clinical endpoints should therefore be interpreted with caution. In order to address the clinical consequences of the increased inflammatory response, a large-scale study is warranted.

4.2 | Perspective

The present study is the first study in cyclists to demonstrate that physical fitness is a determinant of exercise-induced rise in CRP. Our findings may suggest that the beneficial effects of high degree of physical fitness may not only relate to a favorable modulation of basal inflammation but also an improved biological response to physical stress.

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CONFLICT OF INTEREST

Tor Melberg, Øyunn Kleiven, Magnus Bjørkavoll-Bergseth, Jone Selvåg, Bjørn Auestad, Torbjørn Aarsland, Pål Aukrust, and Stein Ørn have no conflicts of interest to declare. Dr Øyvind Skadberg has received lecture fees from Abbott Diagnostics. Dr. Rolf Bergseth is a medical advisor of the North Sea Race. The results from this present study do not constitute endorsement by ACSM. All the results are presented honestly, without fabrication, falsification, or inappropriate data manipulation.

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