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

Short title: Troponins and exercise

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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 hours prior to, and 3 and 24 hours after the race.

Results: Cardiac TnI concentrations increased markedly from baseline [1.9 (1.6-3.0) ng/L] to 3 hours after the race [52.1 (32.4-91.8) ng/L], declining at 24 hours 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 hours after the race [35.6 (24.4-54.4) ng/L], followed by a decline at 24 hours after the race [10.0 (6.9-15.6) ng/L]. The 99th percentile was exceeded at 3 hours 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

1.0 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 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.

2.0 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 (>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 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 Figure 1).

2.2 Data collection

An extensive logistic system was developed to allow a comprehensive 30 min assessment of each of the more than 1000 study subjects 24 hours before the race, and at 3 and 24 hours following the race (Supplementary Figure 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 hours 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 analysed 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 g/L, and the overall 99^{th} 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 99^{th} 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 3h, n=13 at 24h) were given the value 1.6. Subjects with cTnT values below 3 ng/L (n=582 at baseline, n=2 at 3h, n=31 at 24h) 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 (25^{th} percentile - 75^{th} 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 hours 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 ($eGFR_{CKD\text{-}EPI}$), 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.

3.0 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 hours after the race are shown in Figure 1. At baseline, a total of 40.2 % (cTnI) and 58.1 % (cTnT) had cTn values below the limit of detection (Table 1, Figure 1). Cardiac TnI concentrations increased markedly from baseline [1.9 (1.6-3.0) ng/L] to 3 hours after the race [52.1 (32.4-91.8) ng/L], declining at 24 hours 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 hours after the race [35.6 (24.4-54.4) ng/L], followed by a decline at 24 hours 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 hours and 18% at 24 hours following the race. Cardiac TnT values exceeded the 99th percentile (14 ng/L) in 92% of study subjects at 3 hours, and 30% at 24 hours following the race. Delta cTnI between 3 hours post-race and baseline (ΔcTnI 3-0h) was 49.9 (29.4-87.0) ng/L. Delta cTnT (ΔcTnT 3-0h) was 31.7 (20.6-50.1) ng/L. Cardiac troponin values at 24 hours after the race were also higher than baseline levels in virtually all subjects; ΔcTnI 24h-0h: 7.4 (3.7-16.5) ng/L, ΔcTnT 24h-0h: 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 hours after the race, the correlation was closer (3h: rho=0.87, $p<0.001$, 24h: 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). At baseline age, systolic- and diastolic blood pressure, BMI, body weight, waist circumference, Framingham risk score and LDL-levels were positively 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 hours after the race.

Age was inversely associated with the cTnI response at 3 hours after the race, but positively correlated at baseline and 24 hours 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 hours 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 hours after the race. At 3 hours after the race, male participants had significantly higher cTnT but not cTnI values. The number of participants who exceeded the sex-specific 99^{th} percentile at all time-points was similar for both genders (Supplementary Table 2). Female gender remained a significant predictor for higher cTnI 3 hours after the race (B=-0.27, p=0.002), but not for cTnT. At 24 hours after the race, gender was a borderline significant predictor of the cTnT (B=0.09, p=0.096), but not the cTnI response.

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

hours after the race and from baseline to 24 hours 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-hour post-race (B=0.004, p=0.077) and with cTnT 24 hours post-race (B=0.003, p=0.028) were borderline significant. Mean heart rate did not remain a significant predictor for cTnT 3 hour post-race or cTnI 24 hours 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 hours 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 Figure 3). High-Risk individuals had similar levels of cTn at 3 hours after the race and higher cTn levels at 24 hours 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 Figure 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.

4.0 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 more than 84% of subjects exceeding the 99th percentile of the cTn assays at three hours following exercise. At 24 hours 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 hours 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 hours 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 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 (Figure 1, Supplementary Table 2). In multiple regression analysis, however, females were found to have a higher cTnI increase as compared to men at 3 hours 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 hours after the race was identified in this High-risk group ($p<0.01$, Supplementary Figure 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.

5.0 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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6.0 References

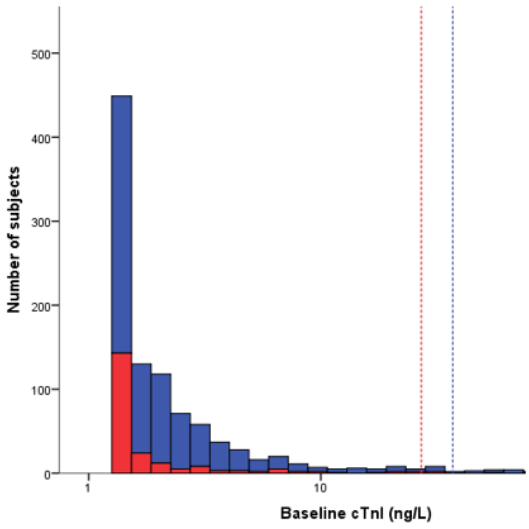
1. Thygesen K, Alpert JS, Jaffe AS et al. Fourth universal definition of myocardial infarction (2018). European heart journal 2018.
2. Gresslien T, Agewall S. Troponin and exercise. International journal of cardiology 2016;221:609-21.
3. Mingels AM, Jacobs LH, Kleijnen VW et al. Cardiac troponin T elevations, using highly sensitive assay, in recreational running depend on running distance. Clinical research in cardiology : official journal of the German Cardiac Society 2010;99:385-91.
4. Shave R, George KP, Atkinson G et al. Exercise-induced cardiac troponin T release: a meta-analysis. Medicine and science in sports and exercise 2007;39:2099-106.
5. Sedaghat-Hamedani F, Kayvanpour E, Frankenstein L et al. Biomarker changes after strenuous exercise can mimic pulmonary embolism and cardiac injury--a metaanalysis of 45 studies. Clinical chemistry 2015;61:1246-55.
6. Mair J, Lindahl B, Hammarsten O et al. How is cardiac troponin released from injured myocardium? European heart journal Acute cardiovascular care 2017:2048872617748553.
7. Weil BR, Suzuki G, Young RF, Iyer V, Canty JM, Jr. Troponin Release and Reversible Left Ventricular Dysfunction After Transient Pressure Overload. Journal of the American College of Cardiology 2018;71:2906-2916.
8. Neilan TG, Januzzi JL, Lee-Lewandrowski E et al. Myocardial injury and ventricular dysfunction related to training levels among nonelite participants in the Boston marathon. Circulation 2006;114:2325-33.
9. Eijsvogels TM, Veltmeijer MT, George K, Hopman MT, Thijssen DH. The impact of obesity on cardiac troponin levels after prolonged exercise in humans. European journal of applied physiology 2012;112:1725-32.
10. Mingels A, Jacobs L, Michielsen E, Swaanenburg J, Wodzig W, van Dieijen-Visser M. Reference population and marathon runner sera assessed by highly sensitive cardiac troponin T and commercial cardiac troponin T and I assays. Clinical chemistry 2009;55:101-8.
11. Fortescue EB, Shin AY, Greenes DS et al. Cardiac troponin increases among runners in the Boston Marathon. Annals of emergency medicine 2007;49:137-43, 143.e1.
12. Scherr J, Braun S, Schuster T et al. 72-h kinetics of high-sensitive troponin T and inflammatory markers after marathon. Medicine and science in sports and exercise 2011;43:1819-27.
13. Eijsvogels TM, Hoogerwerf MD, Maessen MF et al. Predictors of cardiac troponin release after a marathon. Journal of science and medicine in sport 2015;18:88-92.
14. Tanaka H, Monahan KD, Seals DR. Age-predicted maximal heart rate revisited. Journal of the American College of Cardiology 2001;37:153-6.
15. Ungerer JP, Tate JR, Pretorius CJ. Discordance with 3 Cardiac Troponin I and T Assays: Implications for the 99th Percentile Cutoff. Clinical chemistry 2016;62:1106-14.
16. Fu F, Nie J, Tong TK. Serum cardiac troponin T in adolescent runners: effects of exercise intensity and duration. International journal of sports medicine 2009;30:168-72.
17. Roca E, Nescolarde L, Lupon J et al. The Dynamics of Cardiovascular Biomarkers in non-Elite Marathon Runners. Journal of cardiovascular translational research 2017;10:206-208.

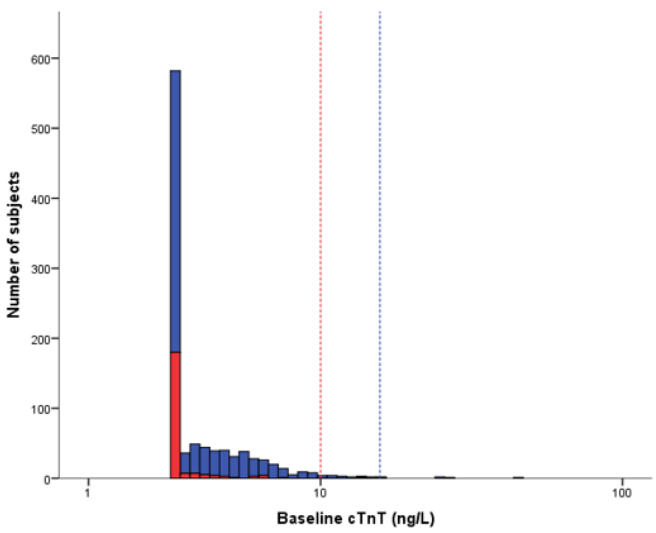
18. Serrano-Ostariz E, Legaz-Arrese A, Terreros-Blanco JL et al. Cardiac biomarkers and exercise duration and intensity during a cycle-touring event. Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine 2009;19:293-9.
19. Kleiven O, Bjorkavoll-Bergseth M, Melberg T et al. High physical fitness is associated with reduction in basal- and exercise-induced inflammation. Scandinavian journal of medicine & science in sports 2018;28:172-179.
20. Stewart GM, Yamada A, Haseler LJ et al. Influence of exercise intensity and duration on functional and biochemical perturbations in the human heart. The Journal of physiology 2016;594:3031-44.
21. Kim YJ, Ahn JK, Shin KA, Kim CH, Lee YH, Park KM. Correlation of Cardiac Markers and Biomarkers With Blood Pressure of Middle-Aged Marathon Runners. Journal of clinical hypertension (Greenwich, Conn) 2015;17:868-73.
22. Park MH, Shin KA, Kim CH et al. Effects of Long-Distance Running on Cardiac Markers and Biomarkers in Exercise-Induced Hypertension Runners: An Observational Study. Annals of rehabilitation medicine 2018;42:575-583.
23. Blackburn H, Jacobs D, Jr. Commentary: Origins and evolution of body mass index (BMI): continuing saga. International journal of epidemiology 2014;43:665-9.
24. Prentice AM, Jebb SA. Beyond body mass index. Obesity reviews : an official journal of the International Association for the Study of Obesity 2001;2:141-7.
25. Sahlen A, Gustafsson TP, Svensson JE et al. Predisposing factors and consequences of elevated biomarker levels in long-distance runners aged >or=55 years. The American journal of cardiology 2009;104:1434-40.
26. Hubble KM, Fatovich DM, Grasko JM, Vasikaran SD. Cardiac troponin increases among marathon runners in the Perth Marathon: the Troponin in Marathons (TRIM) study. The Medical journal of Australia 2009;190:91-3.
27. Scharhag J, Herrmann M, Urhausen A, Haschke M, Herrmann W, Kindermann W. Independent elevations of N-terminal pro-brain natriuretic peptide and cardiac troponins in endurance athletes after prolonged strenuous exercise. American heart journal 2005;150:1128-34.
28. Kong Z, Nie J, Lin H et al. Sex differences in release of cardiac troponin T after endurance exercise. Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals 2017;22:345-350.
29. Borjesson M, Urhausen A, Kouidi E et al. Cardiovascular evaluation of middle-aged/ senior individuals engaged in leisure-time sport activities: position stand from the sections of exercise physiology and sports cardiology of the European Association of Cardiovascular Prevention and Rehabilitation. European journal of cardiovascular prevention and rehabilitation : official journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology 2011;18:446-58.

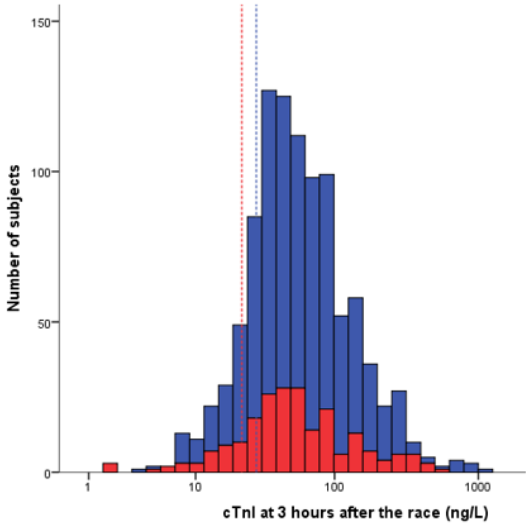
Figure legends

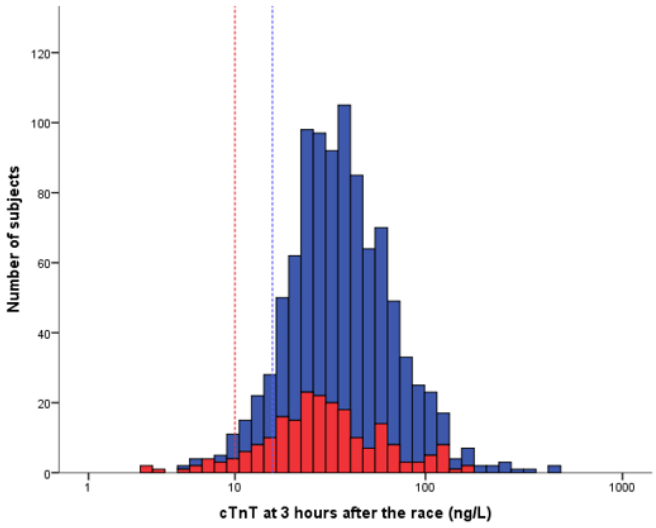
Figure 1

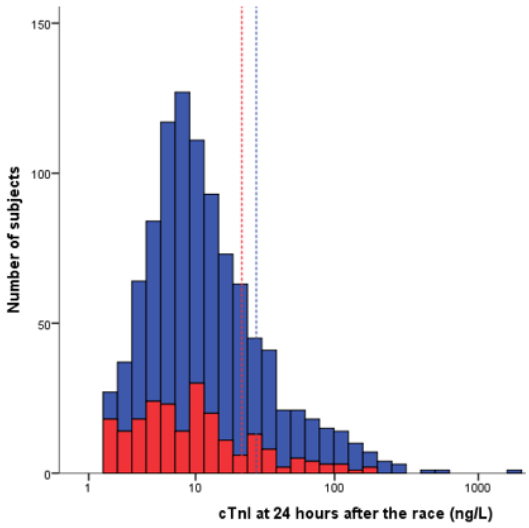
Distribution of cTn at baseline, and at 3- and 24 hours after the race, red indicates female subjects; blue male subjects. Dotted lines represent the sex-specific 99th percentile.

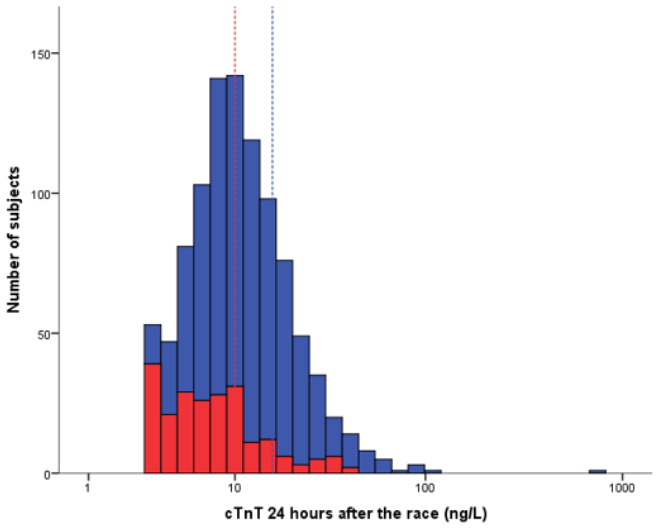

Number of subjects
Baseline cTnI (ng/L)


Number of subjects
Baseline cTnT (ng/L)


Number of subjects
cTnI at 3 hours after the race (ng/L)


Number of subjects
cTnT at 3 hours after the race (ng/L)


Number of subjects
cTnI at 24 hours after the race (ng/L)


Number of subjects
cTnT 24 hours after the race (ng/L)

1

2

Figure 2

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

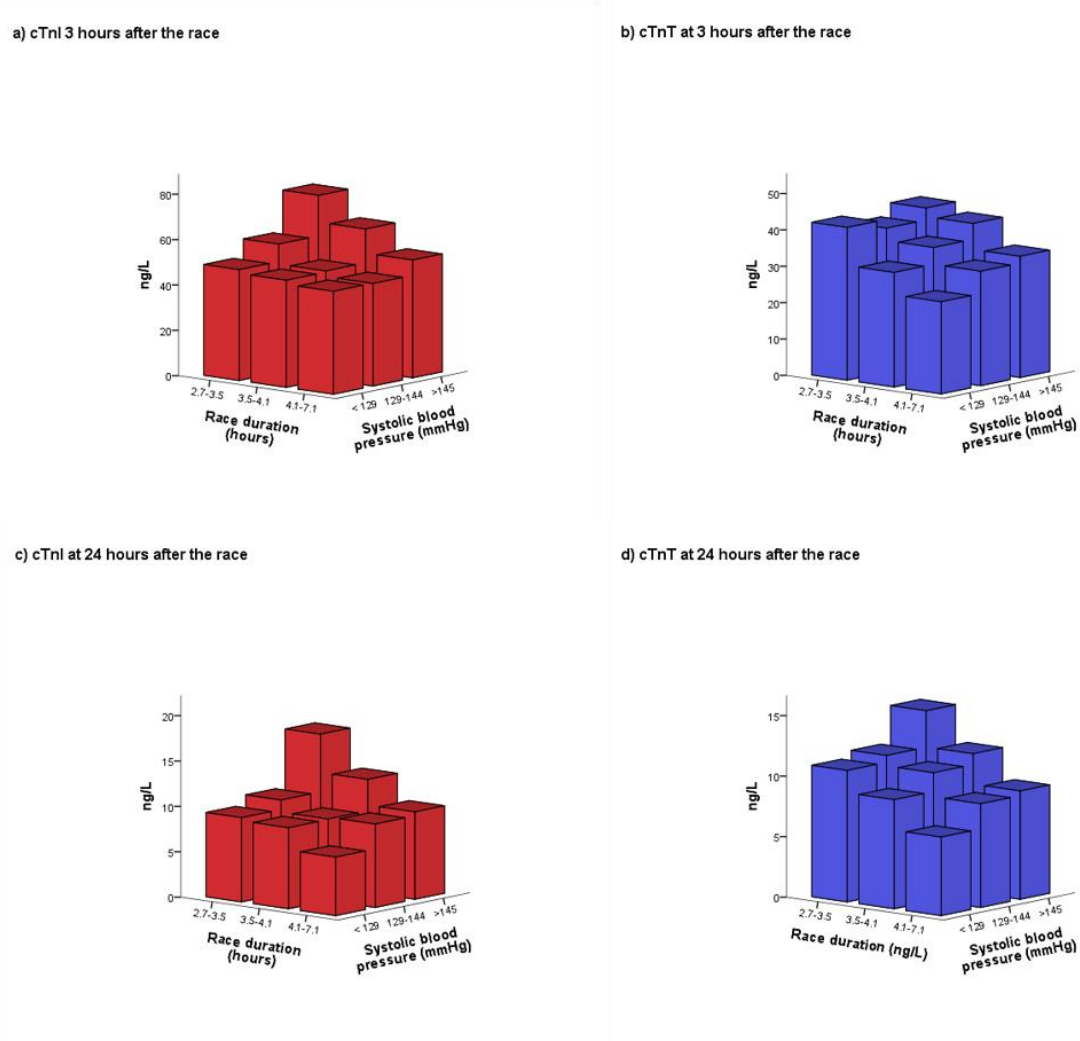


Table 1

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, %[*]	1 (0-5)
Physical fitness	
Resting heart rate, beats/min	59 (53-67)
MET hours per week [†]	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 [‡]	1.9 (1.6-3.0)
cTnT, ng/L [§]	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

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

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-hour post-race				cTnT 3-hour post-race			
	Bivariate correlations		Multiple regression (R^2=0.15)		Bivariate correlations		Multiple regression (R^2=0.16)	
	Rho	p-	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-hour post-race				cTnT 24-hour 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-	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		
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		