Cardiac Troponin T and I Release After a 30-km Run

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Prolonged endurance-type exercise is associated with elevated cardiac troponin (cTn) levels in asymptomatic recreational athletes. It is unclear whether exercise-induced cTn release mirrors a physiological or pathological underlying process. The aim of this study was to provide a direct comparison of the release kinetics of high-sensitivity cTnI (hs-cTnI) and T (hs-cTnT) after endurance-type exercise. In addition, the effect of remote ischemic preconditioning (RIPC), a cardioprotective strategy that limits ischemia-reperfusion injury, was investigated in a randomized controlled crossover manner. Twenty-five healthy volunteers completed an outdoor 30-km running trial preceded by RIPC (4 × 5 min 220 mm Hg unilateral occlusion) or control intervention. hs-cTnT, hs-cTnI, and sensitive cTnI (s-cTnI) concentrations were examined before, immediately after, 2 and 5 hours after the trial. The completion of a 30-km run resulted in a significant increase in circulating cTn (time: all p <0.001), with maximum hs-cTnT, hs-cTnI, and s-cTnI levels of 47 ± 27, 69 ± 62, and 82 ± 64 ng/L (mean ± SD), respectively. Maximum hs-cTnT concentrations were measured in 60% of the participants at 2 hours after exercise, compared with maximum hs-cTnI and s-cTnI concentrations at 5 hours in 84% and 80% of the participants. Application of an RIPC stimulus did not reduce exercise-induced cTn release (time × trial: all p >0.5). In conclusion, in contrast to acute myocardial infarction, maximum hs-cTnT levels after exercise precede maximum hs-cTnI levels. Distinct release kinetics of hs-cTnT and hs-cTnI and the absence of an effect of RIPC favors the concept that exercise-induced cTn release may be mechanistically distinct from cTn release in acute myocardial infarction. © 2016 Elsevier Inc. All rights reserved. (Am J Cardiol 2016;118:281−287)

Insight into the (patho)physiology of exercise-induced cardiac troponin (cTn) release is an active and relevant topic of discussion.[1] The recent advent of both a

high-sensitivity (hs-) assay for cTnI (cTnI) and T (cTnT) with similar analytical characteristics enables to directly compare the kinetics of both diagnostic equivalent molecules. For example in acute myocardial infarction (AMI), where cTn release results from the breakdown of the contractile apparatus after ischemic myocardial injury, peak levels of hs-cTnI and hs-cTnT are reached at similar levels after admission.[2,3] A direct comparison of exercise-induced hs-cTnI and hs-cTnT release could provide more insight into the underlying (patho)physiology. In addition, remote ischemic preconditioning (RIPC), a powerful noninvasive cardioprotective strategy, can be applied to study the contribution of an imbalance between oxygen supply and demand in exercise-induced cTn release. RIPC describes the application of brief episodes of nonlethal ischemia and reperfusion to a tissue or an organ, resulting in protection of the same or another visceral organ against an injurious ischemic insult in the future.[4] In line with the cardioprotective effect of RIPC in various settings of tissue ischemia, we hypothesize that if an oxygen demand-supply imbalance contributes to exercise-induced cTn release, application of an RIPC stimulus would result in reduced postexercise cTn levels. The aim of this study was a head-to-head comparison of the release kinetics of hs-cTnT and hs-cTnI after prolonged endurance-type exercise. In

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addition, we examined the effect of RIPC on exercise-induced cTn release using a randomized controlled single-blind crossover design.

Methods

Twenty-nine healthy runners (age range 18 to 65 years) were recruited with posters at local running clubs. Before testing, all participants were informed about the study procedures and method but remained naïve to the study rationale. This study was carried out according to the principles of the Declaration of Helsinki and approved by the local Institutional Review Board and Ethics Committee of Maastricht University Medical Center. All subjects provided written informed consent before participation. This study was conducted from March 2013 to June 2013 and was registered at clinicaltrials.gov as NCT01774461.

In a randomized controlled single-blind crossover design, participants completed 2 identical outdoor 30-km running trials, either preceded by an RIPC or control intervention. Both experimental test days started in the morning and were separated by at least 2 weeks. Before each trial, participants were instructed to refrain from any strenuous physical labor and sports activities for 24 hours and to standardize their breakfast on both experimental days. The running trials were organized in a research setting on paved (bicycle) lanes with a minimal number of cross sections with the start and finish ≈ 1 km from Maastricht University Medical Center. While running, participants were individually followed by a researcher on a bicycle and allowed to drink water and Isostar sports drink ad libitum. Before the first running trial, a comprehensive 2-dimensional and 3-dimensional transthoratic echocardiogram at rest was recorded from all participants. No abnormalities were observed.

Temporal unilateral upper arm ischemia was achieved by inflating a blood pressure cuff to 220 mm Hg. Circulatory occlusion lasted 5 minutes and was followed by 5 minutes of reperfusion by deflation of the cuff. This sequence was repeated for a total of 4 cycles and resulted in a total procedure time of 40 minutes (4 × [5 minutes of circulatory occlusion + 5 minutes of nonocclusion]). The control intervention followed an identical protocol, except for the blood pressure cuff being inflated to only 20 mm Hg, allowing uninterrupted perfusion. The order of testing (RIPC vs control) was randomized and counterbalanced between participants.

Blood samples were collected in serum and ethylenediaminetetraacetic acid-containing tubes at baseline (preceding the RIPC intervention), immediately after exercise, 2 and 5 hours after exercise. Within 1 hour of collection, hematology parameters were analyzed on a Sysmex XE-5000 (Kobe, Japan) analyzer. In addition, serum tubes were centrifuged, and aliquots were stored at −80°C until analysis. Serum cTnT was measured using the high-sensitivity assay of Roche Diagnostics (Basel, Switzerland); the clinical reference limit (99th percentile of a healthy reference population) is 14 ng/L, with a 10% analytical variation at 13 ng/L.[5] Serum cTnI was measured with the following 2 assays: the STAT high-sensitive troponin I assay (Abbott Diagnostics, Abbott Park, IL) and the Access AccuTnI+3 assay (Beckman Coulter, Brea,

CA) According to the package insert, the 99th percentile limit of the Abbott hs-cTnI assay is 26.2 ng/L with a corresponding coefficient of variation of 4%. The Beckman Coulter assay has a 99th percentile limit of 40 ng/L and a corresponding 10% imprecision, as specified by the manufacturer. Complementary cardiovascular or skeletal biomarkers; N-terminal pro−B-type natriuretic peptide (NTproBNP), creatine kinase (CK), CK muscle and brain fraction (CK-MB), and lactate dehydrogenase were measured using assays of Roche Diagnostics. In addition, creatinine (Roche Diagnostics) and cystatin C (Gentian, Moss, Norway) were measured to calculate the estimated glomerular filtration rate according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[6]

Sample size estimation was based on hs-cTnT as the primary outcome variable. We aimed to include 29 runners to detect a 30% reduction in exercise-induced hs-cTnT release due to RIPC (from 40 ± 32 ng/L to 28 ± 32 ng/L, mean $\pm$ SD) with a statistical power of 0.8 at an alpha error probability of 0.05. An estimated postexercise hs-cTnT concentration of 40 ng/L was based on our previous studies of marathon-induced hs-cTnT release.[7,8] The expected effect size of 30% was derived from the reports, where a 40% reduction of cTn release by RIPC preceding cardiac and vascular surgery has been observed.[9−11] Assuming a similar effect in endurance-type exercise, we considered that a <30% reduction would exclude a relevant contribution of ischemia-reperfusion in the setting of exercise-induced cTn release.

Data are presented as mean $\pm$ SD. A 2-factor (time × trial) repeated-measures general model was used to assess difference in biochemical parameters. In addition, trial order (control or RIPC intervention) was entered into the statistical model as a between-subject variable, partitioning out this source of variability (analyses not shown). In the case of a nonnormal distribution, data were transformed by natural logarithm. All statistical tests were 2-tailed, and a p value of <0.05 was considered statistically significant. Analyses were performed using IBM SPSS Statistics for Windows 22.0 (IBM Corp, Armonk, NY).

Results

Twenty-nine participants (23 men and 6 women) were included in the study. Three male participants left the study prematurely due to a sports-related injury (n = 2) or medical reason unrelated to the study protocol (n = 1) and were therefore not included in the analysis. One male participant was excluded after study completion because of highly elevated hs-cTnT (Roche), hs-cTnI (Abbott), and sensitive cTnI (s-cTnI, Beckman) concentrations (72, 316, and 332 ng/L, respectively) before his second running trial. In the absence of clinical symptoms, these values were highly suggestive for exercise-induced troponin release in the past 24 hours and hence indicative for noncompliance to a critical aspect of the study protocol (refrainment of exercise in the 24 hours preceding the run). Therefore, 25 participants (19 men and 6 women) with an age of 40 ± 13 years (mean $\pm$ SD) were included in the present analysis (Table 1). Subjects were moderately to highly trained, with an average of 52 running kilometers per week (range: 20 to 120).

Variable	Subjects' characteristics
Men	19 (76%)
Age (years)	40 ± 13
Body mass index (kg/m^2)	22 (18-25)
Physical activity (h/week)	7 (2-17)
Running activity (km/week)	52 (20-120)
Running experience (years)	10 (2-30)
Marathon or ultra-distance experience	14 (56%)

Data are presented as n (%), mean ± SD or mean (range).

Fourteen runners (56%) completed at least one marathon or ultradistance trial. None of the subjects had a history of cardiovascular disease, assessed by medical questionnaire.

The exercise trials were separated by a median of 2 weeks (range: 2 to 7). All participants completed both trials within a time span of 1:57:13 to 3:13:36 (hours:minutes:seconds), without symptoms of myocardial ischemia. There was no difference in the completion time between the control trial and the trial preceded by ischemic preconditioning: mean control trial 2:26:56 and mean RIPC trial 2:26:54. The overall average timeframe between the intervention and the 30-km run was 24 minutes (range: 15 to 41) and similar in the setting with and without RIPC. The overall average delay from finish to the first postexercise blood sample collection was 23 minutes (range: 14 to 33) with no differences in the setting with and without RIPC.

At baseline of the control trial, almost all athletes displayed hs-cTnT, hs-cTnI, and s-cTnI concentrations below the clinical decision limit of myocardial infarction (24, 23, and 24 participants, respectively; Figure 1). The completion of a 30-km run resulted in an expected significant increase of circulating hs-cTnT, hs-cTnI, and s-cTnI (time: all p <0.001; Figure 1, Table 2, Supplementary Figure 1). Maximum concentrations of hs-cTnT, hs-cTnI, and s-cTnI were 47 ± 27 ng/L, 69 ± 62 ng/L, and 82 ± 64 ng/L (mean ± SD, control trial) and exceeded the clinical reference value of hs-cTnT, hs-cTnI, and s-cTnI in 100% (n = 24), 60% (n = 15), and 68% (n = 17) of the participants. The kinetics of hs-cTnT was different from hs-cTnI and s-cTnI, as 60% of the participants (n = 15) displayed hs-cTnT maximum concentrations at 2 hours after exercise, whereas maximum concentrations of hs-cTnI and s-cTnI were measured at 5 hours after exercise in 84% and 80% participants (n = 21 and n = 20), respectively. Elevations of hs-cTnT, hs-cTnI, and s-cTnI measured immediately after exercise in the control trial were significantly correlated with each other (Pearson: all R >0.8, p <0.001). In addition, NTproBNP concentrations increased significantly after the 30-km run with maximum concentrations of 14 ± 8 pmol/L (mean ± SD, control trial, time: p <0.001; Figure 1, Table 2, Supplementary Figure 2). Elevations of NTproBNP were not significantly associated with hs-cTnT, hs-cTnI, or s-cTnI (Pearson: all R <0.2, p >0.3).

Application of an RIPC stimulus by 4 cycles of 5-minute blood pressure cuff inflation followed by 5 minutes of deflation did not reduce exercise-induced cTn release at 0, 2, and 5 hours after exercise (Figure 1, Table 2). Similar results were obtained when correcting for plasma volume changes (Supplementary Figure 3). The inclusion of trial order and age of the participants in the model did not affect the results (analyses not shown). In addition, exercise-induced changes of NTproBNP, CK, CK-MB, lactate dehydrogenase, and estimated glomerular filtration rate did not differ between the control and RIPC trial (time × trial: all p >0.1, Table 2).

Discussion

The present study provides a direct comparison of the release kinetics of hs-cTnT and hs-cTnI after prolonged endurance-type exercise. By serial sampling after exercise, we demonstrate that hs-cTnT levels peak earlier than hs-cTnI levels. Furthermore, we show that RIPC, a powerful noninvasive cardioprotective strategy in the setting of ischemia-induced cTn release, has no effect on exercise-induced cTn release.

Recreational running and the participation in long-distance running events, such as (half-) marathons has increased tremendously in recent years. Although the cardiovascular benefits of regular physical exercise are widely established, it is unclear whether this linear dose−benefit response relation is present among the whole spectrum from regular physical exercise to strenuous (ultra) endurance−type exercise.[12] An important issue in this intense scientific debate is the phenomenon of exercise-induced cTn release. This is the first study that measures the release kinetics of both hs-cTnT and hs-cTnI after prolonged endurance-type exercise. In most of the participants, maximum concentrations of hs-cTnT, hs-cTnI, and s-cTnI exceeded the clinical reference value. As cTnT and cTnI are biologically equivalent for the diagnosis of AMI,[13,14] we observed high correlations between all cTn concentrations immediately measured after exercise. However, we demonstrate that hs-cTnT displays maximum concentrations at an earlier stage after exercise compared with hs-cTnI (2 hours vs >5 hours, respectively). This observation is distinct from cTn release resulting from the breakdown of the contractile apparatus after ischemic myocardial injury, where peak levels of hs-cTnT and hs-cTnI are reached within the same time line after hospital admission, followed by cTn elevations for over several days after the onset of myocardial ischemia.[2,3] Although cTnI and cTnT are different molecules measured with different assays, we observed that median maximum postexercise hs-cTnI concentrations were numerically in the same range of hs-cTnT levels (69 ng/L and 47 ng/L, respectively). Remarkably, these concentrations are distinct from cTn release in patients with AMI, where hs-cTnI levels have been measured in multiples of hs-cTnT (range of multiple of hs-cTnI levels compared with hs-cTnT: 3.4 to 12.4).[2,15,16] All together, these observations suggest that exercise-induced cTn release may be mechanistically distinct from irreversible cardiomyocyte injury in AMI.

An aim of this study was to assess the effect of RIPC on exercise-induced cardiac biomarker release. The cardioprotective effect of RIPC has initially been studied in single-center studies of patients undergoing cardiac surgery or angioplasty. Application of RIPC has been shown to significantly reduce postintervention cTn release, which
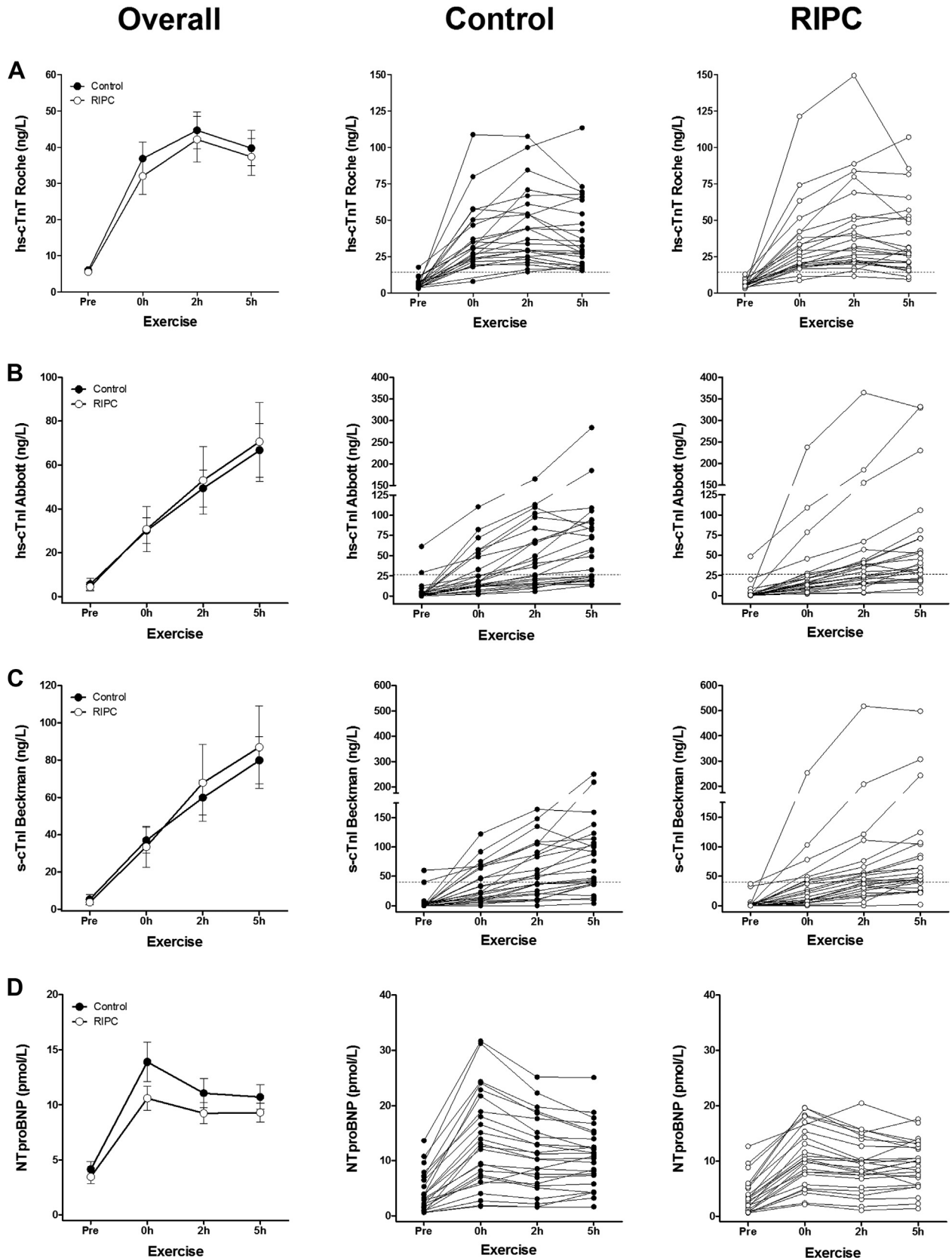
Figure 1. Release of cardiac biomarkers induced by a 30-km run preceded by a control *(closed circles)* or RIPC *(open circles)* intervention. Depicted are the overall and individual concentrations of hs-cTnT *(A)*, hs-cTnI *(B)*, s-cTnI *(C)*, and NTproBNP *(D)*. Overall concentrations are presented as mean ± SEM. The *dotted line* represents the clinical reference value of 14 ng/L (hs-cTnT), 26.2 ng/L (hs-cTnI), and 40 ng/L (s-cTnI).

Table 2
Release of cardiac and skeletal biomarkers induced by a 30-km run preceded by a control or remote ischemic preconditioning intervention

Parameter	Pre-exercise	0h post-exercise	2h post-exercise	5h post-exercise	P-value
hs-cTnT Roche (ng/L)					Time: p <0.001
Control	6 ± 4	37 ± 22	45 ± 26	40 ± 24	Trial: p=0.2
RIPC	6 ± 3	32 ± 25	42 ± 31	37 ± 26	Time x Trial: p=0.5
hs-cTnI Abbott (ng/L)					Time: p<0.001
Control	6 ± 13	30 ± 28	49 ± 42	67 ± 61	Trial: p=0.4
RIPC	4 ± 10	31 ± 51	53 ± 78	71 ± 90	Time x Trial: p=0.5
s-cTnI Beckman (ng/L)					Time: p<0.001
Control	5 ± 14	37 ± 33	60 ± 47	80 ± 63	Trial: p=0.2
RIPC	4 ± 10	33 ± 53	68 ± 104	87 ± 110	Time x Trial: p=0.7
NTproBNP (pmol/L)					Time: p<0.001
Control	4 ± 4	14 ± 9	11 ± 7	11 ± 6	Trial: p=0.1
RIPC	3 ± 3	11 ± 5	9 ± 5	9 ± 4	Time x Trial: p=0.1
CK (U/L)					Time: p<0.001
Control	144 ± 88	267 ± 125	314 ± 127	423 ± 185	Trial: p=0.9
RIPC	129 ± 81	264 ± 132	323 ± 167	505 ± 300	Time x Trial: p=0.1
CK-MB (µg/L)					Time: p<0.001
Control	4 ± 3	6 ± 3	7 ± 3	10 ± 5	Trial: p=0.9
RIPC	4 ± 2	6 ± 3	8 ± 4	12 ± 7	Time x Trial: p=0.2
LD (U/L)					Time: p<0.001
Control	173 ± 26	274 ± 60	259 ± 51	252 ± 45	Trial: p=0.5
RIPC	167 ± 21	276 ± 63	262 ± 58	249 ± 48	Time x Trial: p =0.2
eGFR (mL/min/1.73 m^2)					Time: p<0.001
Control	110 ± 12	79 ± 16	94 ± 15	103 ± 13	Trial: p =0.4
RIPC	108 ± 12	79 ± 20	93 ± 20	100 ± 18	Time x Trial: p=0.6

Data are presented as mean ± SD.

CK = creatine kinase; CK-MB = creatine kinase muscle and brain fraction; eGFR = estimated glomerular filtration rate; hs-cTnT = high-sensitivity cardiac troponin T; hs-cTnI = high-sensitivity cardiac troponin I; LD = lactate dehydrogenase; NTproBNP = N-terminal pro−B-type natriuretic peptide; s-cTnI = sensitive cardiac troponin I; RIPC = remote ischemic preconditioning.

further translated into an improvement in all-cause mortality.[17,18] The exact signaling cascade of RIPC is the subject of intense research, but it is evident that the mediator triggers protection ubiquitously in all visceral organs, by the activation of cryoprotective signaling.[4] However, this beneficial result of RIPC was not observed in 2 recent large-scaled multicenter studies of RIPC, which could be related to a greater number of cardiovascular risk factors and medications in the clinical setting than in patients in the pilot studies.[19,20] We observed no effect of an RIPC stimulus preceding an endurance-type exercise trial on the kinetics of hs-cTnT, hs-cTnI, s-cTnI, and other cardiac or skeletal biomarkers. By the time this study was conducted, a study was published where also no effect of RIPC on hs-cTnI levels induced by a cycling exercise trial was observed.[21] However, no direct comparison of cTnT and cTnI kinetics was performed. In the present study, we applied running as the exercise model and measured both hs-cTnT and hs-cTnI kinetics. Taken together, these findings demonstrate that RIPC has no effect on exercise-induced cTn release.

The biological mechanism underlying exercise-induced cTn release remains unclear, and there is currently limited evidence for a specific mechanism. Several other theories involving the altered environment of cardiomyocytes have been suggested, such as stimulation of integrins related to myocardial stretch or the formation of free oxygen radicals. Similar to findings in the literature, we observed no significant correlation between NTproBNP concentrations

immediately after exercise and hs-cTnT, hs-cTnI, or s-cTnI,[22,23] which does not provide strong evidence for the contribution of myocardial stretch in exercise-induced cTn release. In addition, the role of free oxygen radicals as contributing mechanism is lacking due to the observations that antioxidant supplementation had no effect on exercise-induced cTn release.[24]

Endurance-type exercise training stimulates physiological cardiac adaptations that facilitate appropriate increases in cardiac output during exercise. For example, endurance-trained subjects exhibit mostly eccentric hypertrophy, which is characterized by dilatation of the cardiac chambers and an increase in the maximal wall thickness.[25] The inverse association of training status with postexercise concentrations of cTn,[7,26] as well as an increase in baseline and postexercise cTn after an endurance training program,[27] suggest that elevations of cTn may be linked to structural and functional cardiac adaptations in response to endurance-type exercise. However, this relation between biochemical and functional cardiac changes after endurance-type exercise is currently unclear.

Although our acute findings of endurance-type exercise suggest that the underlying mechanism may be different from irreversible cardiomyocyte injury in AMI, findings of subclinical coronary artery disease, right ventricular fibrotic lesions and right ventricular arrhythmias have been observed among extensively trained (veteran) athletes.[28−30] The susceptibility of individual athletes to these injurious effects of strenuous exercise is not fully

understood, and the role of exercise-induced hs-cTnT and hs-cTnI kinetics in the risk stratification of individual athletes is unclear.

Disclosures

The authors have no conflicts of interest to disclose.

Supplementary Data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j. amjcard.2016.04.030.

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