Exercise-induced release of cardiac troponin is attenuated with repeated bouts of exercise: impact of cardiovascular disease and risk factors

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Abstract

Background: Prolonged exercise can induce cardiac troponin release. Since single bouts of exercise may protect against cardiac injury, we explored the hypothesis that the magnitude of exercise-induced release of troponin attenuates upon successive days of exercise. We also examined whether effects of successive exercise bouts differ between healthy participants and individuals with cardiovascular risk factors (CVRF) and established cardiovascular disease (CVD).

Methods: We examined cardiac troponin I (cTnI) concentrations from whole venous blood samples collected from the antecubital vein (10 mL) in 383 participants (61±14 years) at rest and immediately following 4 consecutive days of long-distance walking (30-50 km/day). Participants were classified as either healthy (n=222), CVRF (n=75) or CVD (n=86).

Results: Baseline cTnI concentrations were significantly higher in CVD and CVRF participants compared to healthy (P<0.001). Exercise-induced elevations in cTnI were observed in all groups following all days of walking compared to baseline (P<0.001). Tobit regression analysis on absolute cTnI concentrations revealed a significant day*group interaction (P=0.04). Following day 1 of walking, post-hoc analysis showed that exercise-induced elevations in cTnI attenuated on subsequent days in healthy and CVRF, but not in CVD. Odds ratios for incident cTnI concentrations above the upper reference limit were significantly higher in comparison to baseline on Day 1 for healthy (4.90 (95% CI 1.58-15.2)) and CVD participants (14.9 (1.86-125)); and remained significantly higher than baseline on all subsequent days in CVD.

Conclusions: The magnitude of post-exercise cTnI concentrations following prolonged walking exercise significantly declines upon repeated days of exercise in healthy individuals and those with CVRF, whilst this decline is not present in CVD patients.

New & Noteworthy: We show the magnitude of post-exercise cardiac troponin concentrations following prolonged walking exercise significantly declines upon repeated days of exercise in healthy individuals and those with cardiovascular risk factors, whilst this decline is not present in patients with established cardiovascular disease.

Key words: cardiovascular risk; exercise training; cardiovascular disease; prevention; preconditioning

Introduction

The performance of exercise leads to an increase in circulating cardiac troponin (cTnI) (1), a powerful and clinically used marker of cardiac injury (2). This exercise-induced increase is moderated by factors including exercise intensity, duration, and health status (3). Since exercise-induced elevations in cTnI is commonly present, even in healthy individuals without cardiac symptoms/events, studies have argued that this reflects a benign response (3). However, studies have demonstrated exaggerated exercise-induced elevations in cTnI in individuals with cardiovascular diseases (4), coronary artery disease (5) and myocardial fibrosis (6).We recently revealed that exercise-induced cTnI elevations above the 99th percentile following prolonged walking exercise in an older, nonathletic population was predictive of future cardiovascular events and mortality (4). These observations highlight the importance to better understand the exercise-induced elevation in cTnI.

Previous studies demonstrated that single or short-term exercise can offer immediate protection against vascular and cardiac injury (7, 8). For example, a single bout of exercise preceding cardiac ischaemia-reperfusion injury affords protection, leading to a smaller infarct size in animal models (8). Interestingly, exercise-induced cTnI release was blunted during a second exercise session timed 48-hours later (9) or following training (10), and declined with successive days of running in humans (11). Whilst these findings support the ability of exercise to attenuate exercise-induced cTnI release, no studies evaluated these effects in groups with elevated risk. Pre-clinical evidence indicates that cardiovascular risk factors (CVRF) and/or cardiovascular disease (CVD) attenuates the efficacy of cardioprotection (12-14). If CVD and CVRF also alter the effects of exercise against post-exercise cTnI release is currently unexplored.

We examined whether 4 successive exercise bouts alter the magnitude and presence of detectable exercise-induced cTnI release, and whether these effects are different between healthy individuals *versus* subjects with cardiovascular risk or disease. We hypothesized that cTnI concentrations will attenuate across 4 successive days of exercise in all three groups due to exercise-induced

cardioprotection from preceding walking days, whilst this decline in exercise-induced cTnI would be attenuated in participants with CVRF and CVD (15).

Methods

Participants

Participants included in this study participated in the Nijmegen Four Days Marches (edition 2009/2010/2014/2015/2016); an annual event that involves 4 consecutive days of walking either 30, 40 or 50 km/day, depending on sex and age, at a self-selected pace and rest times. Participants were recruited via social media and the Nijmegen Marches website and were classified into one of the following 3 groups: healthy, CVRF, or established CVD. Participants in the CVRF group were included if they were diagnosed by a physician and currently were under treatment for hypertension, hypercholesterolemia, and/or diabetes mellitus. Participants classified into the CVD group had a diagnosis of myocardial infarction, stroke, or heart failure. Healthy participants did not have any of the inclusion criteria outlined for participants in the CVRF and CVD group. This study was approved by the medical ethical committee of the Radboud University Medical Center and was conducted in accordance with the Declaration of Helsinki. Written informed consent was provided by all volunteers prior to participation in the study.

Study procedures

Baseline measures took place one (between 9am-5pm) or two (between 12pm-5pm) days before the start of the march and were conducted under controlled, resting conditions. All participants reported to a laboratory, located near the start- and finish-area of the march. Questionnaires related to demographics and health status were provided to all participants in the weeks prior to the walking event. Information related to cardiovascular health status and prescribed medications were used to

categorize volunteers. On the 4 successive walking days, participants reported to our laboratory following finishing (<15-minutes), which was facilitated through the close proximity of our laboratory to the start-/finish area (~100m). Timing of the post-exercise measurements of cardiac troponin was 10-20 minutes following the finish and were collected between 12pm and 5pm.

Measurements

Subject characteristics. Measures of height and weight (Seca 888 scale, Hamburg, Germany) were collected in duplicate, and subsequently body mass index (BMI) was calculated. Body fat percentage was calculated from four-point skinfold thickness (biceps, triceps, sub-scapular, supra-iliac), and this measure was obtained by a single, qualified researcher (16). To determine waist circumference, a measurement was taken midway between the lower rib margin and iliac crest. Following 5-minutes of supine rest, baseline measures of resting heart rate and blood pressure (BP) were measured in duplicate using an automated sphygmomanometer (M5-1 Intellisense, Omron Health Care, Hoofddorp, The Netherlands).

Exercise characteristics. To determine exercise duration and walking speed, start and finish times were recorded following each day of prolonged walking. On Day 1, heart rate was recorded with a 2-channel electrocardiographic chest band system (Polar Electro Oy, Kempele, Finland) and measured with a data recorder every 5km along the route to determine average heart rate. Exercise intensity was calculated as average heart rate during exercise divided by estimated maximum heart rate (208–0.7xage) (17), and is presented as percentage of maximal heart rate (% HR_{max}).

Cardiac troponin I (cTnI) analysis. At baseline and following completion of exercise on Day 1 to 4, venous blood was drawn from the antecubital vein (10 mL). Whole venous blood samples were collected in serum-gel Vacutainer tubes and allowed to clot for ~45-minutes. Following centrifugation of samples, serum was aliquoted, frozen, and stored at −80°C for later analysis. Concentrations of cTnI were

analyzed using a contemporary cTnI assay (ADVIA Centaur TnI-Ultra; Siemens Healthcare Diagnostics, The Hague, The Netherlands) with an established upper reference limit (URL) of 0.040 µg/L, which represents the clinical cut-off values for myocardial infarction (15, 18, 19). The coefficient of variation is 8.8% at the URL and 10% at 0.030 µg/L. The analytical limit of detection (LoD) is 0.006 µg/L. For each day, we present cTnI on a continuous scale (i.e. primary outcome), whilst we also report the prevalence of individuals who report detectable cTnI and those with cTnI concentrations above the URL (>URL; secondary outcome).

Statistical Analysis

Statistical analyses were performed using SPSS Statistics 27 (SPSS, Inc., Chicago, Illinois) and Stata 16.0, and statistical significance was set at $p<0.05$. Continuous variables were reported as mean ± SD and categorical variables as proportions. One-way ANOVA was used to compare baseline characteristics across groups. A Pearson Chi-Square test was used to compare categorical variables at baseline and a post-hoc z-test comparison of columns with Bonferroni's correction was done in case of statistical significance. To assess changes in cTnI concentrations, we used a random effects tobit regression model using log-transformed cTnI concentrations. With the regression model we compared cTnI concentrations at baseline and following each day of prolonged walking ('days'), and evaluated whether changes in cTnI were different between groups ('group', 'days*group'). We added age and sex as covariates in the model since these factors impact baseline and/or exercise-induced cTnI levels (20, 21). Conducting a tobit regression allowed us to model the latent distribution of cTnI concentration, thus accounting for undetectable values below the lower limit. The random effects model also accounted for any missing values. To evaluate secondary endpoints, i.e., proportion of individuals with cTnI above the detection limit (0.006 µg/L) or upper reference limit (>URL: 0.04 µg/L), binomial logistical regression was

161 performed. This allowed us to evaluate the odds ratio (OR) of cTnI levels above the detection limit or
162 URL following each walking day compared to baseline, which we also adjusted for age and sex.
163
164 **Results**
165 Out of the 383 participants (n=246 men, age=21-89 years) who participated, a total of 24 participants
166 dropped out from walking, with n=16 on Day 1 (healthy=7, CVRF=5, CVD=4), n=2 on Day 2 (healthy=1,
167 CVRF=1), and n=6 on Day 3 (healthy=3, CVRF=1, CVD=2). None dropped out because of cardiac-
168 /cardiovascular-related problems and none of the participants reported cardiac symptoms during or
169 following exercise. Beta-blocker use was present in participants with CVD and this group demonstrated
170 a lower HR on Day 1 compared to healthy and CVRF participants (**Table 1**). CVD participants walked for a
171 shorter duration than healthy and CVRF participants ($p<0.05$).. Walking speed was similar on days 1 and
172 3, and was slightly but significantly lower on days 2 and 4 for all groups. Importantly, this difference was
173 marginal (~0.14 km/h) and not different between groups ($p=0.09$) (**Table 2**). CVRF and CVD volunteers
174 were older and thus, covered a shorter distance in comparison to healthy participants (**Table 1**).
175
176 *Baseline.* CVD and CVRF had higher baseline cTnI than healthy participants (tobit regression; $P<0.001$).
177 Chi-square analysis revealed that detectable cTnI-concentrations were more frequent in CVRF and
178 established CVD compared to healthy participants ($P<0.001$; **Table 1**), whilst we found no differences
179 between groups for cTnI concentrations >URL ($P=0.78$; **Table 1**).
180
181 *Exercise and health status.* Regression analysis revealed significant main effects for 'group' ($p<0.001$),
182 'day' ($p<0.001$), and 'day*group' interaction ($p=0.04$). For all groups, cTnI was significantly higher during
183 walking days than baseline (**Figure 1**). Pairwise comparisons revealed differences in cTnI between groups
184 with successive days of walking. Specifically, cTnI-concentrations on Day 3-4 were significantly lower

compared with concentrations on Day 1 in healthy and CVRF groups, whilst in CVD participants cTnI-concentrations remained elevated across days (**Figure 1**).

Binomial logistic regression model revealed a statistically significant decline in ORs for detectable cTnI (adjusted for age and sex) across days in healthy (P=0.002) and CVRF (P=0.001), but not CVD (P=0.44; **Table 3**). Specifically, in healthy and CVRF participants the OR was significant on Days 1-2, but not on Days 3-4 for healthy (**Table 3**). For the OR for cTnI >URL (>0.04 μg/L), we found a significant decline in OR across days for healthy participants (P=0.035) but not in subjects with CVRF (P=0.48 Table 3). In healthy individuals the OR for post-exercise cTnI values >URL was significant on Day 1 only, whereas CVD participants showed an increased OR on all days in the adjusted analysis (P=0.05, **Table 3**).

Discussion

We examined whether successive exercise bouts alter exercise-induced cTnI release, and whether cardiovascular disease and/or risk modulate these responses. First, we show that prolonged moderate-intensity walking increases cTnI-concentrations, regardless of health status. Secondly, repeating the same volume of exercise on 4 successive days lowers the magnitude of exercise-induced cTnI release and prevalence of detectable cTnI-levels in healthy and CVRF participants. Third, participants with established CVD did not demonstrate an attenuation in cTnI-release across successive days of exercise and demonstrates significantly higher odds of cTnI-concentrations above the upper reference limit following exercise. These observations demonstrate that successive days of prolonged exercise is associated with a significant decline in the magnitude and prevalence of detectable cTnI, although these effects were not observed in those with established CVD.

Our observation that successive days of prolonged exercise lowers the exercise-induced release in cTnI supports the concept that single or short-term exercise is associated with an attenuation in (cardiac)

injury upon exposure to the same stimulus. Some previous observations support our findings. Middleton *et al.* (11) assessed troponin release following three consecutive days of moderate-intensity running in athletes, and found a decline in exercise-induced troponin release on subsequent days of running. Our observations also fit in previous work in both humans and animals, which demonstrate that single or short-term periods of exercise attenuates *in vivo* or *in vitro* injury of cardiac or vascular tissue (7, 8).

An important observation is the significantly higher cTnI-concentrations across the various days, including higher proportion with cTnI-release >URL, in CVD patients. This is clinically relevant as cardiac injury and future myocardial events are linked with post-exercise cTnI elevations >URL (4). The lack of attenuation of cTnI-release across consecutive days of exercise in CVD patients fits with previous observations. For example, exercise-induced cTnI concentrations remained elevated in subjects with obstructive coronary artery disease compared to healthy peers, with the latter group demonstrating cTnI concentrations back to baseline within 24h (5). The lack of a decline in cTnI-release in CVD patients in our study may, at least in part, relate to a prolonged post-exercise release of cTnI following Day 1, thereby masking a potential decline on following days. Previous work in animals found that myocardial apoptotic rates, a potential underlying mechanism related to cTnI release, increase with acute exercise. Importantly, these rates are exaggerated in aged and untrained animals; a consequence, in part, of increased preload, oxidative stress, and ischemia (20). Future studies are warranted to better understand the mechanisms explaining the lack of decline in exercise-induced cTnI-release in CVD patients.

Limitations. One limitation is the observations of cTnI-concentrations below the detection limit. To account for this, we have adopted a tobit regression method and have presented the proportion of individuals with values above the detectable limit and URL (4). Observations based on the proportion of

detectable cTnI and >URL reinforce our observations using absolute cTnI-concentrations. Another limitation is that we, due to practical issues related to the start of exercise (i.e., between 4-7AM), only assessed cTnI-levels following exercise. Pre-exercise values of cTnI would allow insight whether cTnI-levels returned to baseline on subsequent days, although such data would be affected by diurnal variation in cTnI (22). A final limitation is that exercise intensity was assessed on Day 1 only, which is relevant since exercise intensity impacts the magnitude of acute exercise-induced troponin release (23). Whether different intensities or duration of exercise interact with our results cannot be extrapolated due to the observational nature of the present study.

In conclusion, repeating the same volume of exercise on 4 successive days attenuates the magnitude of exercise-induced cTnI-concentrations as well as the prevalence of detectable and >URL cTnI-levels in healthy and CVRF participants, but not in those with established CVD. Moreover, we show that CVD patients demonstrated higher cTnI-release across successive days of exercise in comparison to healthy and CVRF groups, with significantly higher odds of having cTnI-concentrations >URL. Future work is required to further understand the potential clinical relevance of these observations and explore its clinical translation.

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342 **Table 1**. Baseline cohort characteristics. *Significantly different from CVRF group [+]Significantly different

343 from healthy group

	Healthy (n=222)	CVRF (n=75)	CVD (n=86)	*P-value*
Age (years)	58±15 (21-93)	65±13 (39-89) [+]	67±9 (43-85) [+]	<0.001
Men, n (%)	118 (53)	49 (63)	79 (90) *[+]	<0.001
Height (m)	1.73±0.09	1.73±0.09	1.76±0.07 *[+]	0.025
Weight (kg)	77.66±15.58	82.73±15.76 [+]	82.71±12.78 [+]	<0.01
BMI (kg/m^2)	25.69±3.98	27.47±3.97 [+]	26.61±3.23	<0.01
Waist circumference (cm)	98.84±8.83	100.83±8.45	99.23±6.58	0.20
Fat (%)	31.83±6.89	33.82±6.04[+]	29.28±5.12 *[+]	<0.001
Lean body mass (kg)	53.13±11.70	56.54±11.90[+]	59.60±9.06[+]	<0.001
MAP (mmHg)	100±12	106±14[+]	101±28	0.03
SBP (mmHg)	136±17	144±20	143±75	0.19
DBP (mmHg)	82±10	87±12[+]	80±11*	<0.001
HR average (bpm)	116±18	112±16	100±17 *[+]	<0.001
Walking distance (km)	38.57±7.46	35.90±6.33[+]	34.20±6.01[+]	<0.001
30 km, n (%)	80 (36)	37 (49)	55 (64) [+]	
40 km, n (%)	94 (42)	32 (43)	27 (31)	
50 km, n (%)	48 (21)	6 (8) [+]	4 (5) [+]	
Exercise intensity (% HR_{max})	69±10	69±10	62±10 *[+]	<0.001
Baseline cTnI (ug/L)	0.009±0.001	0.011±0.001	0.011±0.001	<0.001
Baseline detectable cTnI, n (%)	69 (31)	37 (49) [+]	54 (63) [+]	<0.001
Baseline cTnI >URL, n (%)	4 (2)	2 (3)	1 (1)	0.78

344

345 **Table 2.** Total number of participants, walking characteristics, and frequency and prevalence of
346 detectable cTnI concentrations (i.e., ≥0.006 µg/L), and above the upper reference limit (URL; >0.04 µg/L)
347 at baseline and following days 1-4 of prolonged walking in healthy participants, participants with
348 cardiovascular risk factors (CVRF), and with established cardiovascular disease (CVD). A linear mixed
349 model analysis was performed to assess walking characteristics. *Post hoc significantly different from
350 day 2 and day 4 [+]Significantly different from healthy and CVRF participants
351
352

	Group	**Baseline**	**Day 1**	**Day 2**	**Day 3**	**Day 4**			
Total participants	*Healthy*	222	222	191	189	206			
	CVRF	75	74	55	51	67		***P-value***	
	CVD	86	86	73	72	80	**Group**	**Day**	**Interaction**
Walking duration (min)	*Healthy*		514±92*	526±87	517±94*	542±117	<0.001	<0.001	0.14
	CVRF		505±83*	517±79	496±86*	520±114			
	CVD[+]		477±76*	484±74	474±76*	483±109			
Speed (km/h)	*Healthy*		4.58±0.72*	4.46±0.76	4.56±0.71*	4.37±0.67	0.09	<0.001	0.10
	CVRF		4.35±0.69*	4.25±0.71	4.40±0.69*	4.25±0.65			
	CVD		4.35±0.74*	4.29±0.69	4.48±0.66*	4.36±0.73			
Detectable (≥0.006)	*Healthy, n (%)*	69 (31.1)	104 (46.8)	83 (43.5)	69 (36.5)	70 (34.0)			
	CVRF, n (%)	37 (49.3)	60 (81.1)	40 (72.7)	32 (62.7)	44 (65.7)			
	CVD, n (%)	54 (62.8)	63 (73.3)	51 (70.0)	52 (72.2)	53 (66.3)			
Above URL (>0.04 µg/L)	*Healthy, n (%)*	4 (1.8)	17 (7.7)	5 (2.6)	3 (1.6)	8 (3.9)			
	CVRF, n (%)	2 (2.7)	7 (9.5)	4 (7.3)	2 (3.9)	4 (6.0)			
	CVD, n (%)	1 (1.2)	12 (14.0)	11 (15.1)	6 (8.3)	8 (10.0)			

353

354 **Table 3.** Adjusted (age, sex) odds ratio (OR) values and 95% confidence intervals for presence of cardiac
355 troponin I that is detectable (≥0.006) and above the URL (>0.04 μg/L) in comparison to baseline for
356 participants classified as healthy, with cardiovascular risk factors (CVRF) and established cardiovascular
357 disease (CVD). Grey cells indicate a significant OR.

Adjusted OR values		**Day 1**	**Day 2**	**Day 3**	**Day 4**	***P*-value**
Detectable	***Overall***	2.13 (1.58-2.87)	1.93 (1.42-2.62)	1.52 (1.11-2.07)	1.28 (0.95-1.73)	<0.001
(≥0.006 μg/L)	*Healthy*	1.99 (1.33-2.94)	1.85 (1.23-2.79)	1.38 (0.91-2.09)	1.16 (0.77-1.74)	0.002
	CVRF	4.74 (2.22-10.1)	2.82 (1.30-6.13)	1.73 (0.81-3.67)	2.05 (1.02-4.12)	0.001
	CVD	1.65 (0.86-3.21)	1.55 (0.79-3.05)	1.73 (0.87-3.45)	1.20 (0.63-2.31)	0.44
Above URL	***Overall***	5.95 (2.58-13.7)	5.13 (2.09-12.50)	2.74 (1.02-7.30)	3.50 (1.45-8.47)	<0.001
(>0.04 μg/L)	*Healthy*	4.90 (1.58-15.2)	2.31 (0.59-9.09)	1.37 (0.29-6.41)	2.44 (0.71-8.40)	0.035
	CVRF	3.92 (0.78-19.6)	2.80 (0.48-16.4)	1.43 (0.19-10.7)	2.37 (0.41-13.4)	0.48
	CVD	14.9 (1.86-125)	20.8 (2.60-166)	10.6 (1.22-90.9)	10.4 (1.26-83.3)	0.050

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FIGURE LEGENDS

FIGURE 1. Cardiac troponin I (cTnI) concentrations at baseline and after prolonged walking on Days 1-4 in participants who are healthy, with cardiovascular disease risk factors (CVRF), and established cardiovascular disease (CVD). A tobit regression analysis was performed in N=383 participants (246 men) and results are presented for 'days', 'group' and 'days*group'-interaction. Data is presented as the predicted ln(troponin) mean concentrations with 95% confidence intervals. *Post-hoc significantly different from baseline, $p<0.05$ ^ Significantly different from day 1, $p<0.05$