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

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Article

1 **Exercise-induced release of cardiac troponin is attenuated with repeated bouts**

2 **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

90   cardioprotection from preceding walking days, whilst this decline in exercise-induced cTnI would be

91   attenuated in participants with CVRF and CVD (15).

92

93   **Methods**

94   **Participants**

95   Participants included in this study participated in the Nijmegen Four Days Marches (edition

96   2009/2010/2014/2015/2016); an annual event that involves 4 consecutive days of walking either 30, 40

97   or 50 km/day, depending on sex and age, at a self-selected pace and rest times. Participants were

98   recruited via social media and the Nijmegen Marches website and were classified into one of the

99   following 3 groups: healthy, CVRF, or established CVD. Participants in the CVRF group were included if

100   they were diagnosed by a physician and currently were under treatment for hypertension,

101   hypercholesterolemia, and/or diabetes mellitus. Participants classified into the CVD group had a

102   diagnosis of myocardial infarction, stroke, or heart failure. Healthy participants did not have any of the

103   inclusion criteria outlined for participants in the CVRF and CVD group. This study was approved by the

104   medical ethical committee of the Radboud University Medical Center and was conducted in accordance

105   with the Declaration of Helsinki. Written informed consent was provided by all volunteers prior to

106   participation in the study.

107

108   **Study procedures**

109   Baseline measures took place one (between 9am-5pm) or two (between 12pm-5pm) days before the

110   start of the march and were conducted under controlled, resting conditions. All participants reported to

111   a laboratory, located near the start- and finish-area of the march. Questionnaires related to

112   demographics and health status were provided to all participants in the weeks prior to the walking

113   event. Information related to cardiovascular health status and prescribed medications were used to

114   categorize volunteers. On the 4 successive walking days, participants reported to our laboratory

115   following finishing (<15-minutes), which was facilitated through the close proximity of our laboratory to

116   the start-/finish area (~100m). Timing of the post-exercise measurements of cardiac troponin was 10-20

117   minutes following the finish and were collected between 12pm and 5pm.

118

119   **Measurements**

120   *Subject characteristics.* Measures of height and weight (Seca 888 scale, Hamburg, Germany) were

121   collected in duplicate, and subsequently body mass index (BMI) was calculated. Body fat percentage

122   was calculated from four-point skinfold thickness (biceps, triceps, sub-scapular, supra-iliac), and this

123   measure was obtained by a single, qualified researcher (16). To determine waist circumference, a

124   measurement was taken midway between the lower rib margin and iliac crest. Following 5-minutes of

125   supine rest, baseline measures of resting heart rate and blood pressure (BP) were measured in duplicate

126   using an automated sphygmomanometer (M5-1 Intellisense, Omron Health Care, Hoofddorp, The

127   Netherlands).

128   *Exercise characteristics.* To determine exercise duration and walking speed, start and finish times were

129   recorded following each day of prolonged walking. On Day 1, heart rate was recorded with a 2-channel

130   electrocardiographic chest band system (Polar Electro Oy, Kempele, Finland) and measured with a data

131   recorder every 5km along the route to determine average heart rate. Exercise intensity was calculated

132   as average heart rate during exercise divided by estimated maximum heart rate (208–0.7xage) (17), and

133   is presented as percentage of maximal heart rate (% HR_{max}).

134   *Cardiac troponin I (cTnI) analysis.* At baseline and following completion of exercise on Day 1 to 4, venous

135   blood was drawn from the antecubital vein (10 mL). Whole venous blood samples were collected in

136   serum-gel Vacutainer tubes and allowed to clot for ~45-minutes. Following centrifugation of samples,

137   serum was aliquoted, frozen, and stored at −80°C for later analysis. Concentrations of cTnI were

138   analyzed using a contemporary cTnI assay (ADVIA Centaur TnI-Ultra; Siemens Healthcare Diagnostics,

139   The Hague, The Netherlands) with an established upper reference limit (URL) of 0.040 µg/L, which

140   represents the clinical cut-off values for myocardial infarction (15, 18, 19). The coefficient of variation is

141   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

142   day, we present cTnI on a continuous scale (i.e. primary outcome), whilst we also report the prevalence

143   of individuals who report detectable cTnI and those with cTnI concentrations above the URL (>URL;

144   secondary outcome).

145

146   **Statistical Analysis**

147   Statistical analyses were performed using SPSS Statistics 27 (SPSS, Inc., Chicago, Illinois) and Stata 16.0,

148   and statistical significance was set at $p<0.05$. Continuous variables were reported as mean ± SD and

149   categorical variables as proportions. One-way ANOVA was used to compare baseline characteristics

150   across groups. A Pearson Chi-Square test was used to compare categorical variables at baseline and a

151   post-hoc z-test comparison of columns with Bonferroni's correction was done in case of statistical

152   significance. To assess changes in cTnI concentrations, we used a random effects tobit regression model

153   using log-transformed cTnI concentrations. With the regression model we compared cTnI concentrations

154   at baseline and following each day of prolonged walking ('days'), and evaluated whether changes in cTnI

155   were different between groups ('group', 'days*group'). We added age and sex as covariates in the

156   model since these factors impact baseline and/or exercise-induced cTnI levels (20, 21). Conducting a

157   tobit regression allowed us to model the latent distribution of cTnI concentration, thus accounting for

158   undetectable values below the lower limit. The random effects model also accounted for any missing

159   values. To evaluate secondary endpoints, i.e., proportion of individuals with cTnI above the detection

160   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

185   compared with concentrations on Day 1 in healthy and CVRF groups, whilst in CVD participants cTnI-

186   concentrations remained elevated across days (**Figure 1**).

187   Binomial logistic regression model revealed a statistically significant decline in ORs for detectable cTnI

188   (adjusted for age and sex) across days in healthy (P=0.002) and CVRF (P=0.001), but not CVD (P=0.44;

189   **Table 3**). Specifically, in healthy and CVRF participants the OR was significant on Days 1-2, but not on

190   Days 3-4 for healthy (**Table 3**). For the OR for cTnI >URL (>0.04 μg/L), we found a significant decline in

191   OR across days for healthy participants (P=0.035) but not in subjects with CVRF (P=0.48 Table 3). In

192   healthy individuals the OR for post-exercise cTnI values >URL was significant on Day 1 only, whereas CVD

193   participants showed an increased OR on all days in the adjusted analysis (P=0.05, **Table 3**).

194

195   **Discussion**

196   We examined whether successive exercise bouts alter exercise-induced cTnI release, and whether

197   cardiovascular disease and/or risk modulate these responses. First, we show that prolonged moderate-

198   intensity walking increases cTnI-concentrations, regardless of health status. Secondly, repeating the

199   same volume of exercise on 4 successive days lowers the magnitude of exercise-induced cTnI release

200   and prevalence of detectable cTnI-levels in healthy and CVRF participants. Third, participants with

201   established CVD did not demonstrate an attenuation in cTnI-release across successive days of exercise

202   and demonstrates significantly higher odds of cTnI-concentrations above the upper reference limit

203   following exercise. These observations demonstrate that successive days of prolonged exercise is

204   associated with a significant decline in the magnitude and prevalence of detectable cTnI, although these

205   effects were not observed in those with established CVD.

206

207   Our observation that successive days of prolonged exercise lowers the exercise-induced release in cTnI

208   supports the concept that single or short-term exercise is associated with an attenuation in (cardiac)

209 injury upon exposure to the same stimulus. Some previous observations support our findings. Middleton

210 *et al.* (11) assessed troponin release following three consecutive days of moderate-intensity running in

211 athletes, and found a decline in exercise-induced troponin release on subsequent days of running. Our

212 observations also fit in previous work in both humans and animals, which demonstrate that single or

213 short-term periods of exercise attenuates *in vivo* or *in vitro* injury of cardiac or vascular tissue (7, 8).

214

215 An important observation is the significantly higher cTnI-concentrations across the various days,

216 including higher proportion with cTnI-release >URL, in CVD patients. This is clinically relevant as cardiac

217 injury and future myocardial events are linked with post-exercise cTnI elevations >URL (4). The lack of

218 attenuation of cTnI-release across consecutive days of exercise in CVD patients fits with previous

219 observations. For example, exercise-induced cTnI concentrations remained elevated in subjects with

220 obstructive coronary artery disease compared to healthy peers, with the latter group demonstrating

221 cTnI concentrations back to baseline within 24h (5). The lack of a decline in cTnI-release in CVD patients

222 in our study may, at least in part, relate to a prolonged post-exercise release of cTnI following Day 1,

223 thereby masking a potential decline on following days. Previous work in animals found that myocardial

224 apoptotic rates, a potential underlying mechanism related to cTnI release, increase with acute exercise.

225 Importantly, these rates are exaggerated in aged and untrained animals; a consequence, in part, of

226 increased preload, oxidative stress, and ischemia (20). Future studies are warranted to better

227 understand the mechanisms explaining the lack of decline in exercise-induced cTnI-release in CVD

228 patients.

229

230 *Limitations.* One limitation is the observations of cTnI-concentrations below the detection limit. To

231 account for this, we have adopted a tobit regression method and have presented the proportion of

232 individuals with values above the detectable limit and URL (4). Observations based on the proportion of

233 detectable cTnI and >URL reinforce our observations using absolute cTnI-concentrations. Another

234 limitation is that we, due to practical issues related to the start of exercise (i.e., between 4-7AM), only

235 assessed cTnI-levels following exercise. Pre-exercise values of cTnI would allow insight whether cTnI-

236 levels returned to baseline on subsequent days, although such data would be affected by diurnal

237 variation in cTnI (22). A final limitation is that exercise intensity was assessed on Day 1 only, which is

238 relevant since exercise intensity impacts the magnitude of acute exercise-induced troponin release (23).

239 Whether different intensities or duration of exercise interact with our results cannot be extrapolated

240 due to the observational nature of the present study.

241

242 In conclusion, repeating the same volume of exercise on 4 successive days attenuates the magnitude of

243 exercise-induced cTnI-concentrations as well as the prevalence of detectable and >URL cTnI-levels in

244 healthy and CVRF participants, but not in those with established CVD. Moreover, we show that CVD

245 patients demonstrated higher cTnI-release across successive days of exercise in comparison to healthy

246 and CVRF groups, with significantly higher odds of having cTnI-concentrations >URL. Future work is

247 required to further understand the potential clinical relevance of these observations and explore its

248 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

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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

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352

	Group	Baseline	Day 1	Day 2	Day 3	Day 4		P-value	
							Group	**Day**	**Interaction**
Total participants	Healthy	222	222	191	189	206			
	CVRF	75	74	55	51	67			
	CVD	86	86	73	72	80			
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

358

360   **FIGURE LEGENDS**

361   **FIGURE 1.** Cardiac troponin I (cTnI) concentrations at baseline and after prolonged walking on Days 1-4

362   in participants who are healthy, with cardiovascular disease risk factors (CVRF), and

363   established cardiovascular disease (CVD). A tobit regression analysis was performed in N=383

364   participants (246 men) and results are presented for 'days', 'group' and 'days*group'-

365   interaction. Data is presented as the predicted ln(troponin) mean concentrations with 95%

366   confidence intervals. *Post-hoc significantly different from baseline, $p < 0.05$ ^ Significantly

367   different from day 1, $p < 0.05$

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