

1 **Left Ventricular Function and Cardiac Biomarker Release – The Influence of Exercise**
2 **Intensity, Duration and Mode: A Systematic Review and Meta-Analysis**

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18 **Running Title: Left Ventricular Function and Cardiac Biomarker Release – The**
19 **Influence of Exercise Intensity, Duration and Mode: A Systematic Review and Meta-**
20 **Analysis**

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25 **Abstract**

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27 **Objective:** We performed a systematic review, meta-analysis and meta-regression of exercise
28 studies that sought to determine the relationship between cardiac troponin (cTn) and left
29 ventricular (LV) function. The second objective was to determine how study-level and
30 exercise factors influenced the variation in the body of literature.

31

32 **Data Sources:** A systematic search of Pubmed Central, Science Direct, SPORTDISCUS, and
33 MEDLINE databases.

34

35 **Eligibility Criteria:** Original research articles published between 1997-2018 involving
36 >30mins of continuous exercise, measuring cardiac troponin event rates and either LV
37 ejection fraction (LVEF) or the ratio of the peak early (E) to peak late (A) filling velocity
38 (E/A ratio).

39

40 **Design:** Random-effects meta-analyses and meta-regressions with four a priori determined
41 covariates (age, exercise heart rate [HR], duration, mass).

42

43 **Registration:** The systematic search strategy was registered on the PROSPERO database
44 (CRD42018102176).

45

46 **Results:** Pooled cTn event rates were evident in 45.6% of participants (95% CI = 33.6 –
47 58.2%); however, the overall effect was non-significant ($P>0.05$). There were significant
48 ($P<0.05$) reductions in E/A ratio of -0.38 (SMD = -1.2 , 95% CI [-1.4 , -1.0]), and LVEF of $-$
49 2.02% (SMD = -0.38 , 95% CI [-0.7 , -0.1]) pre to post-exercise. Increased exercise HR was a

50 significant predictor of troponin release and E/A ratio. Participant age was negatively
51 associated with cTn release. There was a significant negative association between E/A ratio
52 with increased rates of cTn release ($P < 0.05$).

53

54 **Conclusions:** High levels of statistical heterogeneity and methodological variability exist in
55 the majority of EICF studies. Our findings show that exercise intensity and age are the most
56 powerful determinants of cTn release. Diastolic function is influenced by exercise HR and
57 cTn release, which implies that exercise bouts at high intensities are enough to elicit cTn
58 release and reduce LV diastolic function. Future EICF studies should 1) utilise specific
59 echocardiographic techniques such as myocardial speckle tracking, 2) ensure participants are
60 euhydrated during post-exercise measurements, and 3) repeat measures in the hours following
61 exercise to assess symptom progression or recovery. It is also recommended to further
62 explore the relationship between aging, training history, and exercise intensity on cTn release
63 and functional changes.

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Key Points:
<ul style="list-style-type: none">• The magnitude of exercise induced reductions in diastolic function is related to troponin event rate.• Higher average exercise heart rates are associated with an increased troponin event rate and greater reductions in diastolic function.• Increased age leads to a lower troponin event rate and reduced average exercise heart rates. This may have important implications for older/veteran athletes participating in prolonged endurance events.

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69 **List of Abbreviations**

70 CI – Confidence intervals

71 CS – Circumferential strain

72 cTn – Cardiac troponin

73 E/A Ratio – Early to late diastolic filling velocity ratio

74 EICF – Exercise induced cardiac fatigue

75 HR – Heart rate

76 LS – Longitudinal strain

77 LV – Left ventricle

78 LVEF – Left ventricular ejection fraction

79 PSE – Prolonged strenuous exercise

80 RS – Radial strain

81 RV – Right ventricle

82 $\dot{V}O_2$ – Oxygen consumption

83 hs-cTnT – High-sensitivity cardiac troponin T

84 hs-cTnI – High-sensitivity cardiac troponin I

85 cTnT – Cardiac troponin T assay

86 cTnI-Beckman - Cardiac troponin I assays manufactured by Beckman Coulter

87 cTnI-Siemens - Cardiac troponin I assays manufactured by Siemens

88

89 **1. Introduction**

90

91 Prolonged strenuous endurance exercise (PSE) is associated with altered cardiac physiology,
92 that often manifests as both transient alterations in cardiac function and detectable levels of
93 biomarkers, most commonly cardiac troponin (cTn) in the peripheral circulation (1). In some
94 instances, acute measurements of systolic and diastolic left ventricular (LV) function
95 following PSE measured via echocardiography are transiently reduced compared to resting
96 values (1-32). This exercise induced cardiac fatigue (EICF) typically persists for 12-48 hours
97 post-exercise before the heart recovers and functional measures return to baseline levels (2-
98 4). Similarly, immediately post-exercise cTn levels in the blood of endurance athletes have
99 been reported to exceed clinical detection thresholds for the diagnosis of acute myocardial
100 infarction (5-8), but typically return to baseline within 72 hours post-exercise (9). Currently,
101 there is no consensus on whether the process of biomarker release and transient reductions in
102 ventricular function is a benign, pathological or adaptive response. The inclusion of follow-
103 up measurements during the recovery process from exercise may elucidate the nature of
104 EICF; however, this is not always included in study design. Frequently, only single
105 measurements immediately post-exercise are performed and progression to the restoration of
106 normal cardiac function following exercise warrants investigation. While often measured
107 together (1, 8, 10-28), the extent of a possible relationship between cTn release and cardiac
108 function is unclear, with the majority of studies that measure both variables reporting no
109 correlations between the two (5, 6). It is also still unclear from individual studies whether the
110 release of cTn indicates persistent functional alterations to the myocardium following
111 exercise (9), and how the time course of exercise-induced cTn release differs from coronary
112 events.

113

114 Of particular relevance to athletes and coaches is the influence of exercise intensity, mode
115 and duration on EICF and cTn release. Both significant and non-significant effects of
116 exercise have been reported following varied exercise modes at both ends of the
117 intensity/duration spectrum (15, 29). Indeed, there is evidence of EICF and cTn release
118 following exercise bouts as short as 30 minutes at high relative intensities (30), and from
119 exercise bouts lasting as long as 10-24 hours (6, 31, 32). However, the between-study event
120 rate and participant responses are varied, with studies rarely reporting declines in all cardiac
121 functional variables and one hundred percent incidence of biomarker release across all their
122 participants. In terms of exercise and participant factors that play a role, Shave and colleagues
123 reported that cTn event rates were higher with increased participant body-mass and lower
124 with increased exercise duration, and that there was no effect of exercise mode (33). In their
125 meta-analysis of LV function pre to post exercise, Middleton and colleagues (4) observed that
126 increased exercise duration resulted in greater reductions in post-exercise left ventricular
127 ejection fraction (LVEF) and no effects of training history, age or body mass.
128 Importantly, exercise intensity has not yet been meta-analysed as a moderator variable in the
129 body of EICF research, although the most recent published papers suggest that cTn release
130 and temporarily reduced diastolic function is more readily triggered by short, high-intensity
131 exercise (34). Systolic function appears to be more resilient to exercise and has mostly been
132 reported to decline following long-duration exercise performed at lower relative intensities,
133 such as Ironman triathlon or ultra-marathon races (9).

134

135 The collective findings of the body of published studies do not demonstrate a clear link
136 between cTn release and EICF, as the results are largely varied. The variation in the findings
137 may be explained by the influence of study and participant level factors, such as the timing of
138 post-exercise data collection, blinding sonographers to the trial conditions or unaccounted for

139 participant dehydration. A systematic review of studies measuring both cTn and EICF
140 variables would provide recommendations for future studies of cardiac function and cardiac
141 biomarker release following exercise (4, 33). Additionally, meta-analysis of exercise
142 characteristics (mode, duration and intensity) as moderators in a meta-regression would
143 provide evidence towards a mechanistic explanation of EICF and biomarker release.
144 Therefore, the aim of this study was to conduct a systematic review and meta-analysis of
145 studies that measured both EICF and cTn following endurance exercise, and to elucidate the
146 influence of both exercise and participant factors.

147

148 **2. Materials and Methods**

149

150 This meta-analysis was performed according to PRISMA guidelines. As such, we conducted
151 a literature search for peer-reviewed, English-language journal articles examining the effects
152 of exercise on both cTn release and cardiac function, as measured by echocardiography. The
153 search strategy was registered on the PROSPERO database prior to being conducted (registry
154 number: CRD420181021760. The full search strategy is shown in Figure 1.

155

156 **2.1 Search Strategy**

157

158 Sources used for this search were SPORTDiscus, PubMed Central, Science Direct and
159 MEDLINE. The key words were *cardiac troponin*, *endurance exercise*, and
160 *echocardiography*. The initial search was undertaken by 2 independent researchers (JD,
161 JOD), who selected the studies according to the search strategy and based on the inclusion

162 and exclusion criteria shown below. After the initial database search, the titles and abstracts
163 of all studies identified by the database search were screened for suitability. The reference
164 lists from published papers were also searched for relevant studies that did not appear in the
165 database search, as well as papers that cited the selected studies were reviewed for inclusion.
166 The most recent search for current publications was conducted in the month of July, 2018.

167

168 **2.2 Inclusion and Exclusion Criteria**

169

- 170 1) Blood and echo measurements taken prior-to exercise and within 1 hour of cessation
171 of exercise. Studies falling outside of this range were excluded. The majority of
172 studies returned in the initial literature search collected blood as close to immediately
173 after exercise. Cardiac troponin is released in phases following exercise, therefore a 1-
174 hour cut-off was imposed to attempt to standardise the release phase.
- 175 2) Two-dimensional echocardiographic measurement of either E/A ratio or LVEF or
176 provided data from which they could be calculated.
- 177 3) Taken venous blood samples for cardiac troponin T or I (cTn) or high-sensitivity cTn
178 T or I.
- 179 4) Reporting the number of positive cTn tests pre and post-exercise.
- 180 5) Studies involving pharmacological or dehydration interventions were excluded.
- 181 6) Only studies involving continuous exercise such as running or cycling were chosen.
- 182 7) Studies involving team sports were excluded.
- 183 8) Available information on exercise mode and participant training status.
- 184 9) For inclusion into the regression analysis, studies must have reported average exercise
185 heart rate (HR), exercise duration, participant age, and mass.

186 2.3 Quality Assessment

187

188 The quality of the selected studies was independently rated by JD and JOD, using a modified
189 Cochrane risk of bias table (see Supplementary Table 1). Any disputes on study suitability or
190 quality were discussed with a third researcher (JW). We used selective reporting (C), other
191 bias (D), blinding of participants and personnel (E), and blinding of outcome assessment (F)
192 as the main domains. Random sequence generation (A) and allocation concealment (B) were
193 not assessed due to the nature of the exercise interventions. Studies were given a positive
194 score if accounting for one of the main domains and negative scores were given when the
195 criteria were not met. Full descriptions of the criteria are shown in the supplementary file.
196 Where studies failed to describe any of the details required by the criteria, we assigned a
197 neutral score when the criteria were not relevant to the study design. The total score was
198 summed and the studies were then ranked into groups of low quality (positive score in <2
199 domains), fair quality (positive score in 2 domains), and good quality (positive score in 2
200 domains). A further criterion was added that demonstrated whether the study had accounted
201 for volume depletion due to dehydration following the exercise bout(s).

202

203 ***Definition of Exercise Intensity.*** When available, the mean participant exercise heart rate
204 (HR) was the parameter chosen to indicate the overall exercise intensity per study. Maximum
205 HRs were not reported frequently enough in the literature to allow for the calculation of mean
206 relative intensities. As all studies that reported HR data recruited trained participants only, in
207 order to normalise the HR data as all exercise bouts involved either competitive or time-trial
208 settings, we made the assumption that the mean HRs reported were reflective of the intensity
209 demanded by the duration of the exercise bout.

210 **2.4 Statistical Analysis**

211

212 **2.4.1 Derivation of outcome statistics:** Data were extracted from the studies independently
213 by the first author. The key variables were E/A ratio and LVEF, with inter-individual SDs, as
214 well as total cTn samples and positive cTn responses in each study. The dichotomous event
215 rate for cTn response was defined as the number of participants exceeding the assay detection
216 limit for the specific assay reported in the study. If available, the average exercise HR,
217 exercise duration, participant age and mass were also extracted. If these data were not
218 available, efforts were made to contact the authors of the paper to obtain them.

219

220 **2.4.2 Pooling of results:** Data were analysed using the statistical software CMA (Biostat,
221 Englewood, NJ). Standardised mean differences (Cohen's *d*) with 95% CI's were computed
222 individually for E/A ratio and LVEF, and event rate was calculated for cTn positive results.
223 The effect sizes were weighted according to the intra-study variability (calculated from
224 reported means and SDs), with larger, more precise studies having a greater weighting on the
225 overall effect size. Separate random-effects meta-analysis were run for each variable using
226 CMA's meta-regression function. This yielded the summary measures, significance levels
227 (*P*), and the between-study heterogeneity (Cochran's *Q* and *I*²) and variability (τ^2).

228

229 **2.4.3 Exploration of publication bias:** Publication bias was assessed via funnel plot
230 inspection, with the expectation that studies would be evenly distributed between both sides
231 of the average effect size, with high-precision studies close to the mean. The individual
232 funnel plots are displayed in the supplementary material (Supplementary Figures 1-4).

233 Quantitative assessments of Kendall's τ and Egger's regression are also reported for all 3
234 effect sizes alongside the funnel plots.

235

236 **2.4.4 Exploration of heterogeneity and meta-regression analyses:** Heterogeneity of the 3
237 effect sizes were assessed by Cochran's Q and I^2 values calculated in the random-effects
238 model. Statistical significance for Q was $p < 0.01$ and I^2 of 25, 50 and 75% were interpreted as
239 small, medium and large degrees of heterogeneity. In the event of significant levels of
240 heterogeneity in any of the meta-analyses, exploratory meta-regression analyses were
241 conducted to determine how participant and exercise characteristics accounted for
242 heterogeneous effect sizes. The residual plots were inspected for suitability before reporting
243 the R^2 value of the fitted models. In the event of non-random residual plots, further variables
244 were added to the model to capture possible significant interactions. All covariates were
245 analysed separately and then together, to assess the individual contribution of each variable.
246 A method of moment's estimator for the between-study covariance matrix in the random
247 effect models meta-regression was used. Two sub-group analysis were conducted, the first
248 assessed troponin assay type vs. troponin event rates and five groups were formed for assay
249 types: high-sensitivity cardiac troponin T and I (hs-cTnT and hs-cTnI; respectively), cardiac
250 troponin T assays (cTnT), and cardiac troponin I assays manufactured by Beckman Coulter
251 and Siemens (cTnI-Beckman and cTnI-Siemens; respectively). The effect of study exercise-
252 mode was also assessed, and three groups were formed, which were running, cycling and
253 multisport. Following the meta-analysis of cTn event-rate, we matched the event rate from
254 each study as a separate continuous moderator variable in the meta-analysis of E/A ratio and
255 LVEF.

256

257 **2.4.5 Sensitivity analysis:** We conducted a sensitivity analysis by removing outlying studies.
258 The duration of exercise in the study by Passaglia et al (32) was twice that of the next longest
259 study and was removed from the analysis during the sensitivity analysis. However, no
260 changes were noted following its exclusion, so it was left in the final analysis.

261

262 **3. Results**

263

264 The database search yielded 223 potential studies and after duplicates were removed 222
265 remained. Studies involving chemical interventions, animal studies or *in vivo* methods were
266 excluded, leaving 63 potential studies, which were further refined via the inclusion and
267 exclusion criteria to 23 studies with 32 data sets (see PRISMA flow chart).

268

269 **3.1 Findings of the Systematic Review**

270

271 With the exceptions of the two studies by Chan-Dewar et al. (19, 20), all studies reported that
272 exercise lead to a significantly reduced LVEF, E/A ratio, or both variables. There were five
273 studies in total that reported significant reductions in both E/A ratio and LVEF (6, 10, 25, 26,
274 27) and the study quality amongst these ranged from fair to good. For the detailed breakdown
275 of the level of control applied in each study see Table 2 in the Supplementary file.

276 It was apparent that only half of the studies in our data set made use of serial measurements
277 of cardiac function and biomarker release into the recovery stages, with the remaining half
278 relying upon single measurements taken immediately post-exercise to draw their conclusions.

279 Of the studies that took serial measurements, three studies (1, 17, 28) performed repeated

280 measures for cTn and echocardiography at 6 hours post; three studies (17, 23, 31) only
281 collected cTn samples at 24 hours, and six studies (1, 10, 11, 14, 24, 35) took both cTn and
282 echocardiography at 24 hours; the study by Whyte et al. (6) took post-exercise blood and
283 echo measurements at 48 hours post-exercise. The longest follow-up measurements were in
284 the study of La Gerche et al., (27) who collected blood and LV functional data one-week
285 post-exercise. Of the 24-hour follow-up measurements, all studies reported participants
286 echocardiographic and cTn values returning to pre-exercise levels in all but one study (31),
287 that reported elevated cTn in nine participants at the 24-hour point. Of the studies that took
288 follow-up measurements at 6 hours post-exercise, E/A ratio and cTn remained elevated above
289 pre-exercise values in three studies (1, 17, 28). Nie et al (26) reported that LV function had
290 returned to baseline within 6 hours post-exercise but cTn remained elevated in the cohort.

291

292 Only four of the 22 studies reported correlations with biochemical data and functional cardiac
293 measurements. However, it is important to note that no studies reported any correlations
294 between standard echocardiographic measurements and cTn release. Aagard and colleagues
295 (21) reported that participants who demonstrated the greatest reductions in resting heart-rate
296 variability also released the most cTn. La Gerche et al. (27) found significant ($P < 0.05$)
297 correlations between cTn release and myocardial deformation ($R = 0.45$) and wall motion
298 abnormalities ($R = 0.77$). Nie and colleagues (26) reported that endocardial strain reductions
299 correlated with the magnitude of cTn release ($R = 0.7$), and Tulloh and colleagues (31)
300 reported immediate post-exercise resting cardiac output attenuated the greatest in the
301 participants with the greatest post-exercise cTn release.

302

303 **3.1.1 Fluid Loss.** The monitoring of body mass loss during exercise did not appear to
304 influence the echocardiographic or cTn data. Significant reductions in echocardiographic
305 parameters were found in both monitored and non-monitored studies, and the detection of
306 E/A ratio and LVEF changes was not influenced by weight loss monitoring.

307

308 **3.1.2 Blinding.** The majority of studies did not blind the sonographer or technician, but these
309 studies were not more likely to report reductions in the echocardiographic parameters after
310 exercise. Three studies (10, 20, 35) reported the previously obtained intra-individual CV of
311 their echocardiographic measurements to demonstrate reliability.

312

313 **3.1.3 Strain Imaging.** There were six studies (1, 15, 19, 20, 23, 27) that reported LV or RV
314 strain and strain rates. These variables were reported in limited and insufficient quantities
315 across the 22 studies to facilitate a meta-analysis of the strain data. Exercise was shown to
316 significantly reduce RV longitudinal strain (LS) in two studies (1, 27) that involved >60
317 minutes of exercise, whereas RV LS was maintained in the study of Stewart et al (15), which
318 involved <60 minutes of exercise. Longitudinal LV strain was significantly reduced in three
319 studies (1, 19, 27), and unaffected following a 6 hour triathlon in the study of Leetmaa et al
320 (23). The participants in this study were elite national-level triathletes and it is worth noting
321 that the reported values were substantially higher than in all other studies (27.5% pre-
322 exercise, 26.8% post). Longitudinal strain was increased following the low-intensity exercise
323 bout in Chan-Dewar et al (20), and maintained following <60 minutes of moderate intensity
324 cycling in the studies of Stewart et al. (15) and Chan-Dewar et al. (19). Stewart and
325 colleagues (15) reported large reductions in radial strain (RS) and a small, significant
326 reduction in circumferential strain (CS), with no concomitant change in RS or CS rates. In

327 contrast, RS and CS rates were increased following the lower intensity exercise in the two
328 studies of Chan-Dewar et al (19, 20). It would appear from these findings that cycling
329 exercise of up to 60 minutes duration at low to moderate-intensities does not affect
330 myocardial strain but high intensity cycling exercise of >60 minutes may negatively impact
331 radial, circumferential and longitudinal strain.

332

333 **3.2 Results of the Meta-Analysis**

334

335 **3.2.1 Tests of Heterogeneity:** All three meta-analyses reported evidence of significant
336 heterogeneity between the studies, justifying our use of the random-effects model.

337 Additionally, in each analysis there were significant Tau squared values that indicated real
338 differences in effect sizes, ruling out the likelihood of sampling or random errors and
339 subsequent meta-regressions were carried out in each case (Table 1).

340

341 **3.2.2 Publication Bias Investigation:** There was no sign of publication bias among the 3
342 separate meta-analyses that were conducted. For each variable the funnel plot demonstrated a
343 symmetrical distribution of SE about the mean that closely followed along the guidelines
344 printed by the CMA software. The funnel plot highlighted the absence of clustering around
345 the mean shown in more precise studies, and we can therefore infer an absence of publication
346 bias among the studies meta-analysed. Further tests to explore publication bias also
347 confirmed this was absent; Egger's regression intercepts were non-significant and Kendall's
348 Tau reported a non-significant negative correlation of study size on logit event rate in each
349 case.

350 **3.2.3 Subgroup Analyses:** There was no significant effect of exercise mode on the
351 magnitude of effect sizes or event rates of the 3 variables. Subgroup analysis for each
352 variable demonstrated significant heterogeneity when using the fixed-effects models
353 therefore the findings of the mixed-effects model were used. The second subgroup analysis
354 for cTn assay types found there was a significant effect of assay type on troponin event rate.
355 The overall test of between subgroup heterogeneity was significant for the 5 assay groups (Q
356 = 11.8, $P = 0.019$).

357

358 **3.2.4 Troponin Event Rates:** The overall event rate, as calculated by the random-effects
359 meta-analysis, for the detection of cTn was 45.6%, but not significant (95% CI [33.6, 58.2%],
360 $P = 0.494$) (Figure 2). By assay type, the mean event rate for cTnT was 40% (95% CI [23.3,
361 57.2], $P = 0.21$, $n = 28$); 87.9% for hs-cTnT (95% CI [79.0, 96.7%], $P = 0.01$, $n = 4$), 49.2%
362 for hs-cTnI (95% CI [26.6, 71.9%], $P = 0.96$, $n = 3$), 71.4% for cTnI-Beckman (95% CI
363 [51.3, 91.5%], $P = 0.58$, $n = 2$), and 31.5% for cTnI-Siemens (95% CI [19.3, 43.7%], $P =$
364 0.048, $n = 5$).

365

366 **3.2.5 E/A Ratio:** Post-exercise E/A values were significantly ($P < 0.001$) reduced over pre-
367 exercise levels, the mean difference was -0.38 (SE = 0.041, 95%CI [-0.376, -0.307]) and the
368 SMD (Cohen's d) was -1.197 (95% CI [-1.401, -0.993]) (Figure 3).

369

370 **3.2.6 Left Ventricular Ejection Fraction:** The mean difference in LVEF between pre and
371 post-exercise values was -2.02% (SE = 0.568, 95%CI [-3.14, -0.91]), $d = -0.44$ (95% CI [-
372 0.74, -0.14]) (Figure 4).

373 3.3 Results of the Meta Regression

374

375 **3.3.1 Troponin Event Rate:** Of the 32 data sets, 22 contained exercise HR and duration data,
376 allowing for comparisons of exercise intensity and duration, as well as participant age and
377 mass for inclusion into the regression analyses. For the reduced sample size of $n=22$, between
378 study variance was similar to the original data set (see Table 1). Following univariate
379 regression analyses, we found that post-exercise positive cTn response rate was influenced by
380 increased exercise HR (intensity) and reduced age. The regression coefficient for HR
381 predicted a 0.12 increase in logit event rate per $1 \text{ b}\cdot\text{min}^{-1}$ increase in HR (Figure 5A).
382 Participant age was negatively associated with logit event rate (Figure 5B). The regression
383 coefficient was -0.10 per increased year of age. Tests for remaining unexplained variance
384 were significant for each covariate ($P < 0.001$), highlighting that cTn event rate still differed
385 among studies reporting similar participant age and HR (Table 1). Using the 22 data sets that
386 reported HR and duration, there was a significant effect of exercise on event rate despite a
387 reduced overall event rate (logit event rate [95%CI] = 0.64 [-1.25, 0.01], $P < 0.05$).

388

389 **3.3.2 E/A Ratio:** Of the 28 data sets containing E/A ratio data, 18 reported participant mass,
390 age, exercise HR, and duration. Additionally, cTn event rate (%) was included as a covariate
391 in the regression analyses. The results of the single covariate analyses can be seen in Table 1.
392 We found that post-exercise positive cTn response rate was influenced by increased exercise
393 HR and increased cTn event rate. The regression coefficient for HR predicted a 0.03
394 reduction in E/A ratio SMD per $1 \text{ b}\cdot\text{min}^{-1}$ increase in HR (Figure 5C). Troponin event rate
395 was negatively associated with E/A ratio, the regression coefficient was -0.01 for every 1
396 percent increase in event rate ($p < 0.05$) (Figure 5D).

397 **3.3.3 Left Ventricular Ejection Fraction:** For the reduced sample size of $n = 20$, between
398 study variance was similar to the original data set (see Table 1). We found no significant
399 interaction of any of the moderator variables on effect size, despite there being potentially a
400 large proportion of the variance explainable by study-level covariates, as indicated by I^2 of
401 80.3.

402

403

404

405

406 Table 1. Univariate and multivariate meta-regression analysis for cardiac troponin and E/A ratio.

Model	Troponin Event Rate (logit)						E/A Ratio					
	<i>n</i>	β (95% CI)	<i>Q</i>	<i>I</i> ²	τ^2	<i>R</i> ²	<i>n</i>	β (95% CI)	<i>Q</i>	<i>I</i> ²	τ^2	<i>R</i> ²
Univariate	22		85.6***	75.5	1.43	-	18	-	35.6***	52.2	0.20	-
<i>HR</i>	22	0.118 (0.061, 0.175)***	59.4***	66.3	0.98	0.31	18	-0.029 (-0.054, 0.004)*	27.67*	42.17	0.13	0.33
<i>Age</i>	22	-0.104 (-0.202, -0.005)*	80.3***	75.1	1.46	0.02	18	0.015 (-0.028, 0.059)	34.55**	53.69	0.21	-0.08
<i>Duration</i>	22	0.0003 (-0.006, 0.007)	84.8***	76.4	2.00	0.00	18	-0.002 (-0.006, 0.002)	34.00	52.94	0.21	-0.06
<i>Mass</i>	22	-0.054 (-0.125, 0.018)	80.83***	75.3	1.49	0.00	18	0.011 (-0.020, 0.042)	34.24*	53.27	0.21	-0.05
<i>Troponin</i>	22						18	-0.010 (0.018-0.002)*	26.80*	40.3	0.12	0.37
Multivariate	18	-22.82 (-33.90,-11.75)	20.60*	36.88	0.51	0.80	18	2.32 (-6.3, 10.95)	23.82	49.63	0.19	0.04
<i>HR</i>		0.16*** (0.08, 0.19)						0.01 (-0.09, 0.1)				
<i>Age</i>		-0.15* (-0.29, -0.04)						-0.02 (-0.07, 0.03)				
<i>Duration</i>		0.02 (0.007, 0.010)						0 (-0.06, 0.06)				
<i>Mass</i>		0.11 (-0.01, 0.17)						-0.01 (-0.02, 0.01)				

407

408 β = meta-regression coefficient, *I*² = percentage of between study variation that is due to heterogeneity, τ^2 = between study variance, *Q*-total = weighted sum of squared
 409 differences between individual study effect size and pooled effect size, *R*² = Proportion of total variance explained by covariate model, negative values analogous to zero. * =
 410 *P* < 0.05, ** = *P* < 0.01, *** = *P* < 0.001

411

412 **4. Discussion**

413

414 This is the first systematic review and meta-analysis to focus on the relationship between
415 functional and biochemical indices of cardiac fatigue and damage. We exclusively analysed
416 studies using the mixed methods of echocardiography and cardiac biomarkers to attempt to
417 elucidate the relationship between them, and whether cTn release results in transient
418 functional decrements (28, 34). To better inform endurance athletes, coaches and sports
419 physicians, we also aimed to explore the relationship between exercise duration, mode and
420 intensity in eliciting EICF and cTn release. The results of this study demonstrate that there is
421 an overall reducing effect of prolonged, strenuous exercise on LV diastolic and systolic
422 function, and confirm Shave and colleagues' finding of a ~50% event rate of cTn release
423 (33). Endurance exercise of durations ranging from 45 to 1440 minutes caused significant
424 reductions in E/A ratio of -0.34 ($d = -1.20$, $P < 0.001$), and LVEF of -2.0% ($d = -0.44$, $P =$
425 0.004). These findings agree with the previous meta-analysis performed by Middleton et al
426 (4), who reported mean reductions in E/A ratio of 0.45 (-0.39, -0.51) and LVEF of -1.95% (-
427 1.03, -2.88%) immediately following endurance exercise of similar durations.

428

429 **4.1 Systematic Review**

430

431 The systematic review of the literature aimed to address the influence of study design on the
432 reported outcome measures and to summarise the general findings of the previous works. A
433 key finding was that very few total studies reported LVEF changes across the 22 studies and
434 this may be a factor of the varied time course of systolic and diastolic functional alterations.
435 In pathological conditions, diastolic functional changes occur before systolic (37) and it is

436 possible that following exercise, any systolic functional changes are realised later and
437 potentially missed by the timing of testing protocols.

438

439 Of relevance to future research is the finding that only ten of the studies took
440 echocardiographic measurements immediately prior to exercise. To account for variations in
441 loading conditions and hydration status that influence echocardiographic measurements, it is
442 recommended that pre-exercise measurements be taken as close as possible to the start of
443 exercise (34). Our finding that over half of the sampled studies made inferences about post-
444 exercise cardiac function from potentially inaccurate pre-exercise values should be a future
445 consideration and may assist in explaining the dearth of previously reported correlations
446 between cTn concentration and functional cardiac parameters. Furthermore, only half of the
447 22 studies performed serial follow-up measurements to assess the recovery of the
448 participants. The remaining eleven studies performed echocardiographic and cTn
449 measurements immediately after exercise, which may have influenced the post-exercise
450 readings through mechanisms such as post-exercise tachycardia, hypotension and volume
451 depletion. Although, to account for such factors a level of control was applied in each study
452 that performed only immediate post-exercise measures, either statistically or via fluid
453 replacement to attempt to restore loading conditions. A single post-exercise blood sample
454 may have missed the peak cTn values, which was reported to occur at 6 hours post-exercise
455 in three of the studies that performed follow-up measures. Two studies (1, 17) also examined
456 the time course of LV functional changes across several time points during the acute recovery
457 period, and only the study by Tian et al. (17) reported significant reductions in LVEF at the
458 6-hour post-exercise time point, that were not evident immediately after exercise. Therefore,
459 it is apparent that the quality of half of the reviewed studies suffered from not incorporating

460 multiple time points during the post-exercise data collection periods and by not performing
461 echocardiographic assessments closer to the beginning of exercise.

462

463 **4.2 Meta-Analysis**

464

465 **4.2.1 Effect of Exercise Intensity**

466

467 We found that exercise HR was the strongest predictor of cTn event rate ($R^2 = 0.31$). This
468 current finding of increased cTn event rates with increasing exercise HR supports the theory
469 that cTn release is intensity-dependent and the notion that the rate and force of myocyte
470 contraction influences cTn release. It has been suggested that post-exercise cTn release
471 occurs due to membrane damage caused by increased mechanical force of cardiac
472 myofibrillar contraction (34). In the absence of ischaemia, the transport of intact cTn
473 molecules is potentially mediated by excessive stretch of myofibrils stimulating integrin-
474 mediated transport (38). Troponin I degradation in the absence of ischaemia has been shown
475 to increase with LV preload in the rat model (39). Additionally, increased membrane
476 permeability resulting from oxidative damage and inflammation subsequent to intense
477 exercise may allow cTn release (40). Whether this cTn is bound to the myocardium or stored
478 in the cytoplasm remains to be determined. Figure 6 presents a plausible mechanism for
479 troponin transport from the myocardium to the peripheral circulation. It is therefore plausible
480 that both intact and degraded cTn products found in serum post-exercise originate from
481 viable cardiomyocytes, due to the increased preloads generated during exercise.

482

483 If thresholds of exercise intensity, rather than duration exist at which cTn is released, these
484 may be altered following positive cardiac adaptations that establish improved fitness levels.
485 Unfortunately, it was not possible to separate the cases based on training status in the present
486 study owing to a lack of data. Whether cTn release following exercise is an adaptive response
487 that elicits improved cardiac function and a reduced threshold for future cTn release remains
488 to be thoroughly examined.

489

490 Higher exercise HRs increased the magnitude of the post-exercise alteration in LV diastolic
491 filling. In most cases, this was due to a reduction in peak early (E) and maintenance of peak
492 late (A) transmitral filling velocity, which is associated with increased heart rates and reduced
493 ventricular filling times (34). Potential mechanisms responsible for altered LV relaxation, in
494 addition to prolonged elevated HRs, include downregulation of cardiac β -adrenoceptors
495 mediated by elevated catecholamines during exercise (42). In fact, circulating catecholamines
496 are responsible for maintaining tachycardia during endurance exercise and Breuer et al. (41)
497 have shown that concentrations of plasma catecholamines increase with exercise HR.
498 Alterations in adrenergic responsiveness following exercise were reported by Eysmann and
499 colleagues (42) in both Ironman athletes and healthy sedentary individuals, and these changes
500 were correlated with declines in LV function. However, it remains to be seen how differential
501 modulation of the autonomic nervous system, prolonged heart rates, and substrate circulation
502 factor in the development of EICF (36, 42, 43). Our data support the hypothesised
503 relationship between altered myocardial relaxation and decreased sensitivity of β -
504 adrenoceptors induced by increasing circulating catecholamines ubiquitous to higher exercise
505 HRs during prolonged strenuous exercise (44, 45).

506

507 **4.2.2 Effect of Exercise Mode**

508

509 Our sub group analyses did not identify any differences between exercise mode in contrast to
510 the findings of Shave et al. (33) who reported that running stimulated greater cTn release
511 compared to cycling. While the fixed effects model returned a significant effect of exercise
512 mode, our accompanying tests of heterogeneity did not fulfil the assumptions of the model. It
513 has been hypothesised that running exercise elicits higher HRs than cycling due to greater
514 $\dot{V}O_2$ requirements, recruitment of the upper body musculature and accessory muscles, and the
515 lack of postural support during cycling (46). Additionally, modal differences in cTn release
516 may be explained by attaining higher absolute cardiac and metabolic work rates during
517 running (46, 47). In our data, average cycling HR was 150 vs. 156 b·min⁻¹ in running. It is not
518 possible to compare with the previously published meta-analysis as HR data were unavailable
519 in the studies analysed by Shave and colleagues (33).

520

521 **4.2.3 Effect of Exercise Duration**

522

523 We found no significant effect of exercise duration on either cTn release, LV systolic or
524 diastolic function. When the exercise protocols employed in PSE studies typically involve a
525 maximal race-effort, exercise duration dictates exercise intensity. Previous meta-analyses
526 have failed to investigate the role of exercise HR and have concentrated on duration;
527 therefore, no comparable data exists. The absence of a significant effect of exercise duration
528 on either cTn release or EICF contradict the meta-analyses by Shave et al. (33) and
529 Middleton et al. (4) despite inclusion of a similar amount of studies, cases and participant

530 characteristics. The previous meta-analysis of cTn event rates did not report regression
531 coefficients for significant moderators and also used a fixed-effects meta-regression. This
532 may not have been appropriate, depending on the level of heterogeneity between studies in
533 the model as we encountered significant heterogeneity at the study level in all 3 meta-
534 analyses. In our review, this meant that the true effect size likely differed between studies at
535 similar levels and use of the fixed-effects model would not be appropriate.

536

537 **4.2.4 Participant Age**

538

539 We found that participant age was negatively associated with troponin event rate and we also
540 observed a significant ($P<0.05$) negative correlation between age and HR, indicating that
541 older participants do not achieve higher HRs during exercise. The attainment of higher levels
542 of cardiac work during exercise may therefore be a crucial role in the development of
543 exercise troponin release, as previously indicated. Conversely, the level of training previously
544 achieved may be responsible for reduced cTn event rates with increased age, and it may be
545 that lifelong athletes develop greater thresholds to cTn release than younger, less-trained
546 counterparts. Without training history data, this phenomenon cannot be investigated *via* meta-
547 analytical methods.

548

549 **4.2.5 Troponin Event Rate Affects Diastolic Function**

550

551 Our data demonstrated a significant relationship between the E/A ratio and cTn release,
552 wherein the reduction in E/A ratio was related to the magnitude of cTn positive event rates.

553 This finding is contradictory to the majority of research as few individual studies have
554 reported correlations between any LV functional indices and cTn release. When loading
555 conditions such as resting heart rate and plasma volume are controlled for, exercise induced
556 diastolic functional changes are likely to be caused by reductions in myocardial relaxation.
557 While the mechanistic basis for altered relaxation are yet to be fully determined, increased
558 cardiomyocyte membrane permeability has been suggested as a factor in this, as well as cTn
559 release (9). Although individual studies rarely find significant correlations between the two
560 (12), it may be the case that this was due to insufficient sample sizes to bear out the
561 underlying relationships and the larger sample sizes meta-analysed in this study addressed
562 this limitation.

563

564 **4.3 Limitations**

565

566 Inconsistent timing of the post-exercise echocardiograms and blood samples across the range
567 of studies may have had severe effects on the values we analysed. Mechanisms responsible
568 for this include the time-course of cTn release, which is thought to peak at approximately
569 3hrs post-exercise (12). To control for this variability, we selected studies that obtained blood
570 values within 1hr of the cessation of exercise, and it was apparent from the findings of the
571 systematic review that only a limited amount of studies performed follow-up measurements
572 beyond 1 hour after exercise. Additionally, an important limitation was the inclusion of
573 studies that measured both troponin I and T. Troponin I is thought to be less sensitive than
574 troponin T due to several manufacturers producing assays for it (33). Our sub-group analysis
575 to determine a significant effect of assay type on troponin event rate found that there were
576 significant differences between all assay types, yet there was significant variation and

577 heterogeneity among the 5 sub-groups. The intrinsically high levels of variation amongst the
578 sample sizes in terms of study design, exercise characteristics, and participant variables
579 therefore mean that the influence of assay type on event rate prevalence may come secondary
580 to other such factors. Previous studies measuring both high sensitivity troponin T and I have
581 demonstrated the assays to perform comparably in diagnostic performance in a clinical
582 population, and these are currently the recommended assay-type for cardiac damage
583 diagnosis (48). Echocardiographic variables may be influenced by the timing of post-exercise
584 echocardiograms as loading conditions are altered by blood plasma shifts during and after
585 exercise. Further, the influence of post-exercise hypotension and augmented autonomic
586 modulation during the recovery from exercise should not be overlooked. Stewart and
587 colleagues (1) demonstrated by using more sensitive echocardiographic techniques that when
588 loading and functional requirements are equal to pre-exercise levels, demonstrably greater
589 differences in LV and RV function exist post-exercise. Additionally, the lack of availability
590 of RV data is a key limitation to the implications that can be made from our data, as this
591 chamber has shown to be more susceptible to fatigue in athletes (27).

592 While we found evidence of a significant effect of cTn release with altered diastolic filling,
593 our data are merely correlational. There is insufficient physiological data present in the
594 original studies to determine a causal relationship. In addition, a comprehensive approach in
595 the assessment of diastolic function is required in future research to confirm whether diastolic
596 dysfunction is present in athletes following exercise, including tissue Doppler imaging, left
597 atrial volume, LV twist/untwist velocities, and global strain and strain rate imaging to support
598 this association (1).

599

600

601 **4.4 Conclusion**

602

603 The novel findings of the meta-analysis established exercise intensity, measured *via* average
604 absolute exercise HR, as the predominant factor in causing reduced diastolic function and
605 cTn release. Our finding supports the theory that cTn release and EICF are intensity-
606 dependent as our data did not show a significant effect of duration on any of the cardiac
607 variables. The second novel finding was that increased troponin event rates were positively
608 associated with greater reductions in mitral E/A ratio, which supports the theory that EICF
609 and cTn release are related. The low to moderate R^2 values related to exercise HR and cTn
610 event rate (0.33 and 0.37; respectively. See Table 1) leave a remaining portion of the
611 calculated variance requiring further exploration. Future work should aim to investigate these
612 relationships further, using more specific tissue Doppler measurements of diastolic function,
613 and potentially by employing standardised exercise challenges to normalise cardiac function
614 before and after PSE (1).

615

616 Finally, our finding of a significant negative effect of participant age on cTn event rates
617 indicates that longer-term athletes may be less prone to cTn release as a result of cardiac
618 adaptations pursuant to life-time training volumes (49), challenging the theory that cTn
619 release is related to permanent cardiac injury (50). Future research into the effects of aging on
620 cardiac adaptation to exercise may wish to investigate the characteristics of cTn release
621 following exercise in life-long endurance athletes compared to younger, highly-trained
622 athletes.

623

624

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626

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634

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812 **Figure Legends**

813

814 **Figure 1:** PRISMA flow chart.

815

816 **Figure 2:** Forest plot showing logit event rate (solid squares) and 95% CI limits (solid
817 horizontal lines) for each study. The studies are ranked with ascending magnitude of event
818 rate, by exercise mode sub-groups. The overall random-effects logit event rate is shown in the
819 bottom row (solid diamond). The – symbol indicates data that were unavailable. A logit event
820 rate of 0 corresponds to an event rate of 50%, a negative logit event rate indicates a frequency
821 of troponin release from less than 50% of the participants within the study.

822

823 **Figure 3:** Forest plot showing standardised mean differences (SMD, Cohen's *d*) between pre
824 and post exercise values of the E/A ratio (early to late peak transmitral velocity flow) (solid
825 squares) and 95% CI limits (solid horizontal lines) for each study. The studies are ranked with
826 ascending magnitude of event rate, by exercise mode sub-groups. The overall random-effects
827 SMD is shown in the bottom row (solid diamond). The – symbol indicates data that were
828 unavailable.

829

830 **Figure 4:** Forest plot of standardised mean differences (SMD, Cohen's *d*) between pre/post
831 exercise ejection fraction (solid squares) and 95% CI limits (solid horizontal lines) for each
832 study. The studies are ranked with ascending magnitude of event rate, by exercise mode sub-
833 groups. The overall random-effects logit event rate is shown in the bottom row (solid
834 diamond). The – symbol indicates data that were unavailable.

835

836 **Figure 5:** Random-effects univariate meta-regression **A)** HR on troponin event rates showing
837 the positive relationship between increased exercise intensity and troponin release, **B)**
838 Participant age on troponin event rates showing the negative relationship between increased
839 age and troponin release, **C)** Average HR on E/A ratio SMD (*d*) showing greater reductions
840 in E/A ratio occur as exercise HR increases, **D)** Percentage of participants per study who
841 exceeded the troponin assay limit of detection vs the standardised differences in E/A ratio (*d*)
842 following exercise, indicating that E/A ratio is reduced to a greater extent in studies with
843 higher rates of troponin release. Each circle represents a study and the size of the circle
844 reflects the influence of that study on the model. The regression prediction is represented by
845 the solid line.

846

847 **Figure 6:** Exercise intensity and age influence myocardial diastolic function and cardiac
848 troponin release.

849

850 **Table 1:** Univariate and multivariate meta-regression analyses for cardiac troponin and E/A
851 ratio.