1 **Left Ventricular Function and Cardiac Biomarker Release – The Influence of Exercise**

2 **Intensity, Duration and Mode: A Systematic Review and Meta-Analysis**

3

4 Donaldson, J.A[1]., Wiles, J.D[1]., Coleman, D.A[1]., Papadakis, M[2]., Sharma, R[3]., and O'Driscoll,

5 J.M[1,3]

6

7 [1]School of Human and Life Sciences, Canterbury Christ Church University, Kent, CT1 1QU.

8

9 [2]Cardiology Clinical and Academic Group, St George's University of London, UK.

10

11 [3]Department of Cardiology, St George's Healthcare NHS Trust, Blackshaw Road, Tooting,

12 London, SW17 0QT.

13

14 Correspondence to Dr Jamie O'Driscoll, School of Human and Life Sciences, Canterbury

15 Christ Church University, North Holmes Road, Canterbury, Kent, CT1 1QU. Email:

16 jamie.odriscoll@canterbury.ac.uk; Telephone: 01227782711.

17

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**

21 **Word Count:** 4728

22

23

24

25 **Abstract**

26

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

significant predictor of troponin release and E/A ratio. Participant age was negatively

associated with cTn release. There was a significant negative association between E/A ratio

with increased rates of cTn release ($P < 0.05$).

Conclusions: High levels of statistical heterogeneity and methodological variability exist in

the majority of EICF studies. Our findings show that exercise intensity and age are the most

powerful determinants of cTn release. Diastolic function is influenced by exercise HR and

cTn release, which implies that exercise bouts at high intensities are enough to elicit cTn

release and reduce LV diastolic function. Future EICF studies should 1) utilise specific

echocardiographic techniques such as myocardial speckle tracking, 2) ensure participants are

euhydrated during post-exercise measurements, and 3) repeat measures in the hours following

exercise to assess symptom progression or recovery. It is also recommended to further

explore the relationship between aging, training history, and exercise intensity on cTn release

and functional changes.

Key Points:
• 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.

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

1. Introduction

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

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.

2.3 Quality Assessment

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

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

2.4 Statistical Analysis

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

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

2.4.3 Exploration of publication bias: Publication bias was assessed via funnel plot inspection, with the expectation that studies would be evenly distributed between both sides of the average effect size, with high-precision studies close to the mean. The individual 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).

3.3 Results of the Meta Regression

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

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

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

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

| | Troponin Event Rate (logit) | | | | | | E/A Ratio | | | | | |
Model	n	β (95% CI)	Q	I^2	τ^2	R^2	n	β (95% CI)	Q	I^2	τ^2	R^2
Univariate	22		85.6***	75.5	1.43	-	18	-	35.6***	52.2	0.20	-
HR	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
Age	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
Duration	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
Mass	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
Troponin	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
HR		0.16*** (0.08, 0.19)						0.01 (-0.09, 0.1)				
Age		-0.15* (-0.29, -0.04)						-0.02 (-0.07, 0.03)				
Duration		0.02 (0.007, 0.010)						0 (-0.06, 0.06)				
Mass		0.11 (-0.01, 0.17)						-0.01 (-0.02, 0.01)				

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 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. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

4. Discussion

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

4.1 Systematic Review

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

20

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 ß-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 ß-

504   adrenoceptors induced by increasing circulating catecholamines ubiquitous to higher exercise

505   HRs during prolonged strenuous exercise (44, 45).

506

4.2.2 Effect of Exercise Mode

Our sub group analyses did not identify any differences between exercise mode in contrast to the findings of Shave et al. (33) who reported that running stimulated greater cTn release compared to cycling. While the fixed effects model returned a significant effect of exercise mode, our accompanying tests of heterogeneity did not fulfil the assumptions of the model. It has been hypothesised that running exercise elicits higher HRs than cycling due to greater

$\dot{V}O_2$ requirements, recruitment of the upper body musculature and accessory muscles, and the lack of postural support during cycling (46). Additionally, modal differences in cTn release may be explained by attaining higher absolute cardiac and metabolic work rates during running (46, 47). In our data, average cycling HR was 150 vs. 156 b·min^{-1} in running. It is not possible to compare with the previously published meta-analysis as HR data were unavailable in the studies analysed by Shave and colleagues (33).

4.2.3 Effect of Exercise Duration

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

4.4 Conclusion

601

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

649

650

651

652

653

654

655

656

657

658

Reference List

1. Stewart GM, Yamada A, Haseler LJ, Kavanagh JJ, Chan J, Koerbin G, et al. Influence of exercise intensity and duration on functional and biochemical perturbations in the human heart. J Physiol. 2016;594(11):3031-44.
2. Dawson E, George K, Shave R, Whyte G, Ball D. Does the human heart fatigue subsequent to prolonged exercise? Sports Med. 2003;33(5):365-80.
3. McGavock JM, Warburton DER, Taylor D, Welsh RC, Quinney HA, Haykowsky MJ. The effects of prolonged strenuous exercise on left ventricular function: A brief review. Heart & Lung. 2002;31(4):279-94.
4. Middleton N, Shave R, George K, Whyte G, Hart E, Atkinson G. Left ventricular function immediately following prolonged exercise: A meta-analysis. Med Sci Sports Exerc. 2006;38(4):681-7.
5. Rifai N, Douglas PS, O'Toole M, Rimm E, Ginsburg GS. Cardiac troponin T and I, echocardiographic [correction of electrocardiographic] wall motion analyses, and ejection fractions in athletes participating in the Hawaii Ironman Triathlon. Am J Cardiol. 1999;83(7):1085-9.
6. Whyte GP, George K, Sharma S, Lumley S, Gates P, Prasad K, et al. Cardiac fatigue following prolonged endurance exercise of differing distances. Med Sci Sports Exerc. 2000;32(6):1067-72.
7. Neumayr G, Pfister R, Mitterbauer G, Maurer A, Gaenzer H, Sturm W, et al. Effect of the "Race Across The Alps" in elite cyclists on plasma cardiac troponins I and T. Am J Cardiol. 2002;89(4):484-6.
8. Shave RE, Dawson E, Whyte G, George K, Ball D, Gaze DC, et al. Evidence of exercise-induced cardiac dysfunction and elevated cTnT in separate cohorts competing in an ultra-endurance mountain marathon race. Int J Sports Med. 2002;23(7):489-94.
9. Shave R, Baggish A, George K, Wood M, Scharhag J, Whyte G, et al. Exercise-induced cardiac troponin elevation: evidence, mechanisms, and implications. J Am Coll Cardiol. 2010;56(3):169-76.
10. Middleton N, Shave R, George K, Whyte G, Simpson R, Florida-James G, et al. Impact of repeated prolonged exercise bouts on cardiac function and biomarkers. Med Sci Sports Exerc. 2007;39(1):83-90.
11. Dawson EA, Shave R, George K, Whyte G, Ball D, Gaze D, et al. Cardiac drift during prolonged exercise with echocardiographic evidence of reduced diastolic function of the heart. Eur J Appl Physiol. 2005;94(3):305-9.
12. George K, Whyte G, Stephenson C, Shave R, Dawson E, Edwards BEN, et al. Postexercise Left Ventricular Function and cTnT in Recreational Marathon Runners. Med Sci Sports Exerc. 2004;36(10):1709-15.
13. Scharhag J, Shave R, George K, Whyte G, Kindermann W. "Exercise-induced increases in cardiac troponins in endurance athletes: a matter of exercise duration and intensity?". Clin Res Cardiol. 2008;97(1):62-3.
14. Stewart GM, Kavanagh JJ, Koerbin G, Simmonds MJ, Sabapathy S. Cardiac electrical conduction, autonomic activity and biomarker release during recovery from prolonged strenuous exercise in trained male cyclists. Eur J Appl Physiol. 2014;114(1):1-10.
15. Stewart GM, Yamada A, Haseler LJ, Kavanagh JJ, Koerbin G, Chan J, et al. Altered ventricular mechanics after 60 min of high-intensity endurance exercise: insights from exercise speckle-tracking echocardiography. Am J Physiol Heart Circ Physiol. 2015;308(8):H875-83.
16. Wyatt F, Pawar G, Kilgore L. Exercise Induced Cardiac Fatigue Following Prolonged Exercise in Road Cyclists. ICHPER-SD JR. 2011;6(2):61-6.
17. Tian Y, Nie J, Huang C, George KP. The kinetics of highly sensitive cardiac troponin T release after prolonged treadmill exercise in adolescent and adult athletes. J Appl Physiol (1985). 2012;113(3):418-25.
18. Shave R, Dawson E, Whyte G, George K, Gaze D, Collinson P. Altered cardiac function and minimal cardiac damage during prolonged exercise. Med Sci Sports Exerc. 2004;36(7):1098-103.
19. Chan-Dewar F, Gregson W, Whyte G, Gaze D, Waterhouse J, Wen J, et al. Do the effects of high intensity 40 km cycling upon left ventricular function and cardiac biomarker during recovery vary with time of day? J Sports Sci. 2013;31(4):414-23.

713   20.   Chan-Dewar F, Gregson W, Whyte G, King J, Gaze D, Carranza-Garcia LE, et al. Cardiac
714 electromechanical delay is increased during recovery from 40 km cycling but is not mediated by
715 exercise intensity. Scand J Med Sci Sports. 2013;23(2):224-31.
716   21.   Aagaard P, Sahlen A, Bergfeldt L, Braunschweig F. Heart rate and its variability in response
717 to running-associations with troponin. Med Sci Sports Exerc. 2014;46(8):1624-30.
718   22.   Neilan TG, Januzzi JL, Lee-Lewandrowski E, Ton-Nu TT, Yoerger DM, Jassal DS, et al.
719 Myocardial injury and ventricular dysfunction related to training levels among nonelite participants in
720 the Boston marathon. Circulation. 2006;114(22):2325-33.
721   23.   Leetmaa TH, Dam A, Glintborg D, Markenvard JD. Myocardial response to a triathlon in
722 male athletes evaluated by Doppler tissue imaging and biochemical parameters. Scand J Med Sci
723 Sports. 2008;18(6):698-705.
724   24.   Lucia A, Serratosa L, Saborido A, Pardo J, Boraita A, Moran M, et al. Short-term effects of
725 marathon running: no evidence of cardiac dysfunction. Med Sci Sports Exerc. 1999;31(10):1414-21.
726   25.   Serrano Ostariz E, López Ramón M, Cremades Arroyos D, Izquierdo Álvarez S, Catalán Edo
727 P, Baquer Sahún C, et al. Post-exercise left ventricular dysfunction measured after a long-duration
728 cycling event. BMC Res Notes. 2013;6:211-.
729   26.   Nie J, George KP, Tong TK, Tian Y, Shi Q. Effect of repeated endurance runs on cardiac
730 biomarkers and function in adolescents. Med Sci Sports Exerc. 2011;43(11):2081-8.
731   27.   La Gerche A, Connelly KA, Mooney DJ, MacIsaac AI, Prior DL. Biochemical and functional
732 abnormalities of left and right ventricular function after ultra-endurance exercise. Heart.
733 2008;94(7):860-6.
734   28.   Wilson M, O'Hanlon R, Prasad S, Oxborough D, Godfrey R, Alpendurada F, et al. Biological
735 markers of cardiac damage are not related to measures of cardiac systolic and diastolic function using
736 cardiovascular magnetic resonance and echocardiography after an acute bout of prolonged endurance
737 exercise. Br J Sports Med. 2011;45(10):780-4.
738   29.   Serrano-Ostariz E, Legaz-Arrese A, Terreros-Blanco JL, Lopez-Ramon M, Cremades-
739 Arroyos D, Carranza-Garcia LE, et al. Cardiac biomarkers and exercise duration and intensity during
740 a cycle-touring event. Clin J Sport Med. 2009;19(4):293-9.
741   30.   Middleton N, George K, Whyte G, Gaze D, Collinson P, Shave R. Cardiac troponin T release
742 is stimulated by endurance exercise in healthy humans. J Am Coll Cardiol. 2008;52(22):1813-4.
743   31.   Tulloh L, Robinson D, Patel A, Ware A, Prendergast C, Sullivan D, et al. Raised troponin T
744 and echocardiographic abnormalities after prolonged strenuous exercise-the Australian Ironman
745 Triathlon. Br J Sports Med. 2006;40(7):605-9.
746   32.   Passaglia DG, Emed LG, Barberato SH, Guerios ST, Moser AI, Silva MM, et al. Acute
747 effects of prolonged physical exercise: evaluation after a twenty-four-hour ultramarathon. Arq Bras
748 Cardiol. 2013;100(1):21-8.
749   33.   Shave R, George KP, Atkinson G, Hart E, Middleton N, Whyte G, et al. Exercise-induced
750 cardiac troponin T release: a meta-analysis. Med Sci Sports Exerc. 2007;39(12):2099-106.
751   34.   Shave R, Oxborough D. Exercise-induced cardiac injury: evidence from novel imaging
752 techniques and highly sensitive cardiac troponin assays. Prog Cardiovasc Dis. 2012;54(5):407-15.
753   35.   Shave R, Dawson E, Whyte G, George K, Nimmo M, Layden J, et al. The Impact of
754 Prolonged Exercise in a Cold Environment upon Cardiac Function. Med Sci Sports Exerc.
755 2004;36(9):1522-7.
756   36.   Douglas PS, O'Toole ML, Miller WDB, Reichek N. Different effects of prolonged exercise on
757 the right and left ventricles. J Am Coll Cardiol. 1990;15(1):64-9.
758   37.   Dougherty AH, Naccarelli GV, Gray EL, Hicks CH, Goldstein RA. Congestive heart failure
759 with normal systolic function. Am J Cardiol. 1984;54(7):778-82.
760   38.   Hessel MHM, Atsma DE, van der Valk EJM, Bax WH, Schalij MJ, van der Laarse A. Release
761 of cardiac troponin I from viable cardiomyocytes is mediated by integrin stimulation. Pflugers Archiv.
762 2008;455(6):979-86.
763   39.   Feng J, Schaus BJ, Fallavollita JA, Lee TC, Canty JM, Jr. Preload induces troponin I
764 degradation independently of myocardial ischemia. Circulation. 2001;103(16):2035-7.
765   40.   Scherr J, Braun S, Schuster T, Hartmann C, Moehlenkamp S, Wolfarth B, et al. 72-h kinetics
766 of high-sensitive troponin T and inflammatory markers after marathon. Med Sci Sports Exerc.
767 2011;43(10):1819-27.

768 41.   Breuer HW, Skyschally A, Schulz R, Martin C, Wehr M, Heusch G. Heart rate variability and
769 circulating catecholamine concentrations during steady state exercise in healthy volunteers. Br Heart
770 J. 1993;70(2):144-9.
771 42.   Eysmann SB, Gervino E, Vatner DE, Katz SE, Decker L, Douglas PS. Prolonged exercise
772 alters beta-adrenergic responsiveness in healthy sedentary humans. J Appl Physiol (1985).
773 1996;80(2):616-22.
774 43.   Ashley EA, Kardos A, Jack ES, Habenbacher W, Wheeler M, Kim YM, et al. Angiotensin-
775 converting enzyme genotype predicts cardiac and autonomic responses to prolonged exercise. J Am
776 Coll Cardiol. 2006;48(3):523-31.
777 44.   Douglas PS, O'Toole ML, Katz SE. Prolonged exercise alters cardiac chronotropic
778 responsiveness in endurance athletes. J Sports Med Phys Fitness. 1998;38(2):158-63.
779 45.   Welsh RC, Warburton DER, Humen DP, Taylor DA, McGavock J, Haykowsky MJ.
780 Prolonged strenuous exercise alters the cardiovascular response to dobutamine stimulation in male
781 athletes. J Physiol. 2005;569(Pt 1):325-30.
782 46.   Millet GP, Vleck VE, Bentley DJ. Physiological differences between cycling and running:
783 lessons from triathletes. Sports Med. 2009;39(3):179-206.
784 47.   Schneider DA, Pollack J. Ventilatory threshold and maximal oxygen uptake during cycling
785 and running in female triathletes. Int J Sports Med. 1991;12(4):379-83.
786 48.   Sou SM, Puelacher C, Twerenbold R, Wagener M, Honegger U, Reichlin T, et al. Direct
787 comparison of cardiac troponin I and cardiac troponin T in the detection of exercise-induced
788 myocardial ischemia. Clin Biochem. 2016;49(6):421-32.
789 49.   Scharhag J, Schneider G, Urhausen A, Rochette V, Kramann B, Kindermann W. Athlete's
790 heart. J Am Coll Cardiol. 2002;40(10):1856-63.
791 50.   Wilson M, O'Hanlon R, Prasad S, Deighan A, Macmillan P, Oxborough D, et al. Diverse
792 patterns of myocardial fibrosis in lifelong, veteran endurance athletes. J Appl Physiol (1985).
793 2011;110(6):1622-6.
794 51.   Clarke MS, Caldwell RW, Chiao H, Miyake K, McNeil PL. Contraction-induced cell
795 wounding and release of fibroblast growth factor in heart. Circ Res. 1995;6:927-934.
796 52.   Goette A, Bukowska A, Dobrev D, et al. Acute atrial tachyarrthymia induces angiotensin II
797 type 1 receptor-mediated oxidative stress and microvascular flow abnormalities in the ventricles. Eur
798 Heart J. 2009;30(11):1411-20.
799
800
801
802
803
804
805
806
807
808
809
810
811

Figure Legends

Figure 1: PRISMA flow chart.

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

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

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

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

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

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