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THE ACUTE IMPACT OF CHANGES TO HAEMODYNAMIC LOAD ON THE LEFT VENTRICULAR STRAIN-VOLUME RELATIONSHIP IN YOUNG AND OLDER MEN

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Short title: ε-volume loop detects afterload increase

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30   **ABSTRACT**

31   **Objectives**. Chronic changes in left ventricular (LV) haemodynamics, for example induced by

32   increased afterload (i.e., hypertension), mediate changes in LV function. This study examined

33   the proof-of-concept that: *1*. the LV longitudinal strain (ε)-volume loop is sensitive to detect an

34   acute increase in afterload, and *2*. these effects differ between healthy young *versus* older men.

35   **Methods.** Thirty-five healthy male volunteers were recruited including 19 young (24±2 yr) and

36   16 older participants (67±5 yr). Tests were performed prior to, during and after 10-min recovery

37   from acute manipulation of afterload. Real-time haemodynamic data were obtained and LV

38   longitudinal ε-volume loops were calculated from 4-chamber images using 2D-

39   echocardiography.

40   **Results.** Inflation of the anti-G-suit resulted in an immediate increase in heart rate, blood

41   pressure and systemic vascular resistance, and a decrease in stroke volume (all P<0.05). This

42   was accompanied by a decrease in LV peak ε, slower slope of the ε-volume relationship during

43   early diastole, increase in uncoupling (i.e., compared to systole; little change in ε per volume

44   decline during early diastole and large changes in ε per volume decline during late diastole) (all

45   P<0.05). All values returned to baseline levels after recovery (all P>0.05). Manipulation of

46   cardiac haemodynamics caused comparable effects in young *versus* older men (all P>0.05).

47   **Conclusions.** Acute increases in afterload immediately change the diastolic phase of the LV

48   longitudinal ε-volume loop in young and older men. This supports the potency of the LV

49   longitudinal ε-volume loop to provide novel insights into *dynamic* cardiac function in humans

50   *in vivo.*

51

52   **KEYWORDS:** cardiac adaptation, ε-volume loop, haemodynamics, echocardiography,

53   ultrasound

INTRODUCTION

The ability to provide sufficient cardiac output is constantly challenged by beat-to-beat changes in haemodynamic loading (preload, afterload), contractility and heart rate.[8, 15, 23] Acute changes in left ventricular (LV) haemodynamics and heart rate will alter LV mechanics[1, 4, 5, 14, 17, 24], such as LV peak longitudinal strain (ε)[4, 14, 24], circumferential ε [1, 4, 14, 24], and peak torsion[5], and thus LV function. Chronic exposure to such changes can lead to LV remodeling affecting both LV structure and function.[15] However, current measures for LV function, such as LV peak longitudinal ε, LV ejection fraction (LVEF) or mitral flow velocities all provide only partial insights of the effects on LV function.

In a series of studies, we introduced a novel echocardiographic-based method to provide insight into *dynamic* LV function, using non-invasive simultaneous measures of longitudinal ε and volume (i.e. LV longitudinal ε-volume loop) across a cardiac cycle.[10, 11, 13, 18] Concomitant ε-volume analysis provides insight in the relative contribution of longitudinal ε to volume change during systole and diastole, and thus mechanical contribution to changes LV function. We previously observed that a chronic increase in afterload, induced by aortic stenosis, is associated with less coherence between the systolic and diastolic ε-volume relation, indicating a shift in mechanical (i.e. longitudinal ε) contributions to volume change in either systole or diastole. [10] This highlights the ability of the LV longitudinal ε-volume loop to provide novel and potentially clinically relevant insight into both systolic and diastolic dynamic LV function in response to chronic (patho)physiological stimuli affecting haemodynamics in humans *in vivo*. Whether this technique is also able to detect changes in dynamic LV function upon acute changes in afterload and thus contributes to a better understanding of (patho)physiological effects of various stimuli on the heart is currently unknown.

79 Ageing is known to affect cardiac structure and function(6, 12) and is associated with increased

80 ventricular stiffness(19) as well as reduced ventricular compliance.(7) Ageing is therefore

81 associated with impaired cardiac responses to physiological stimuli such as exercise.(22)

82 Accordingly, older age may influence cardiac responses to acute changes in LV workload.

83 Whether older age alters the shape of the LV longitudinal ε-volume loop or contributes to a

84 different response of the LV longitudinal ε-volume loop to acute changes in LV

85 haemodynamics is yet to be determined.

86

87 First, this study explores the impact of an acute increase in LV afterload on the coherence

88 between systolic and diastolic dynamic LV function (i.e. LV longitudinal ε-volume loop) in

89 healthy volunteers (Aim 1). In line with our previous observation of chronic elevation in

90 afterload(10, 11), we expect that an acute increase in afterload results in less coherence between

91 the systolic and diastolic phase of the LV longitudinal ε-volume loop. Second, we examined

92 the hypothesis that participants of older age will show less coherence between the systolic and

93 diastolic phase of the LV longitudinal ε-volume loop at baseline compared to younger

94 participants, whilst this lack of coherence will not affect the ability to detect an acute increase

95 in LV afterload in older participants (Aim 2).

96

97

98 **METHODS**

99 *Ethics approval*

100 Ethics approval was obtained from the Radboud University Medical Center ethics committee

101 to perform the proposed work (reference number 2016-3166). This study was registered at the

102 Netherlands Trial Register (NTR6349). This study conforms to the standards set by the latest

103 revision of the Declaration of Helsinki.

104

Study population

Forty-two healthy male participants were recruited within the social environment of the study team and colleagues, including 20 young participants (24±2 yr) and 22 older participants (67±5 yr). All participants received a standardized screening protocol, consisting of measurements of blood pressure, weight, height, 12-lead ECG and a health questionnaire. Participants were excluded in the presence of diagnosed hypertension (or usage of antihypertensive medication), diabetes mellitus, the use of medication which influences cardiovascular function, ECG abnormalities or in case of a history of or presently existing cardiovascular diseases. A medical doctor cleared all patients prior to inclusion, taking into account the likelihood of present hypertension and a history of, or present cardiovascular diseases. Before final inclusion all participants provided written informed consent. Additional data regarding the included study population can be found in Table 1.

Study design

A within-subject design was adopted to explore the impact of changes in LV afterload upon dynamic LV function (Aim 1). In addition, a between-subject design was adopted to explore the impact of age upon dynamic LV function at baseline as well as after acute increase in afterload (Aim 2). Assessment was performed prior to, during and after recovery from an acute increase in afterload. To increase LV afterload, an anti-gravity suit (ANTI-G Garment Cutaway CSU-13B/P; a garment fitted with inflatable bladders, capable of applying pressure on the abdomen and lower extremities) was used.(16) The anti-G suit predominantly increases afterload at inflation pressure >~70 mmHg. Nonetheless secondary adaptive mechanisms may also simultaneously influence preload and cardiac contractility(16), as often observed when manipulating cardiac haemodynamics.

129

130 Due to instability after inflation of the anti-G suit in left-lateral supine position, all

131 measurements were performed supine position. Participants were zipped into the anti-G-suit,

132 placed at an echo table and instructed to relax for approximately 10 minutes to allow the

133 haemodynamic system to adjust to the supine position. Hereafter a series of baseline

134 measurements were performed (Phase 1). Subsequently, the anti-G suit was inflated to a

135 pressure 10 mmHg above systolic blood pressure (SBP). After 1-minute a second series of

136 measurements was performed (Phase 2). The anti-G suit was then cautiously deflated and a

137 second resting period of 10 minutes was applied to allow the haemodynamic system to recover.

138 Subsequently, a third series of measurements was performed (Phase 3) to determine whether

139 haemodynamics and echocardiographic measures normalize after a recovery period and thus

140 relate to inflation of the anti-G suit. The participant was then disconnected from all devices and

141 given the necessary time to recuperate.

142

143 *Procedures*

144 *Central haemodynamics.* The Nexfin HD monitor (BMEYE, Amsterdam, The Netherlands)

145 was used to obtain data on the central haemodynamical changes that occurred between the 3

146 measurement phases. Estimates of blood flow, blood pressure and vascular resistance were

147 obtained, using an inflatable finger cuff connected to the left index finger. The data were stored

148 locally and analysed with MATLAB R2014b (Matworks Inc., Massachusetts, United States).

149 For all 3 phases, periods of 5 seconds (time-matched to the echocardiographic data) were

150 extracted from the Nexfin data. The mean values of these 5 second periods were used to define

151 the heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial

152 pressure (MAP), systemic vascular resistance (SVR) and an estimate of contractility (dP/dT).

153

154   *LV volumes and cardiac output.* Echocardiographic data were obtained using a Vivid E9

155   ultrasound machine (GE Medical System, Horton, Norway) with a 1.5-4 MHZ phased array

156   transducer. The data were stored in raw DICOM format in a local archive of the Department of

157   Physiology at the Radboud University Medical Center (Nijmegen). Echocardiographic images

158   were acquired in accordance with the recommendations of the American Society of

159   Echocardiography (ASE) by a single experienced researcher from the RadboudUMC

160   (Nijmegen, the Netherlands) with the patient in the supine position. Standard apical 4, 2 and 3

161   chamber views were collected as well as a flow velocity measurement in the aortic outflow tract

162   (used to determine valve closure time). Images were optimized using gain, compression and

163   reject to ensure adequate endocardial delineation. Frame-rates were standardized at 56 fps and

164   a focal zone was positioned at mid-cavity to reduce the impact of beam divergence.

165

166   Data were analysed using commercially available software (EchoPac version 113.05, GE

167   Medical, Horten, Norway). LV end diastolic volume (LVEDV), LV end systolic volume

168   (LVESV), LV stroke volume (LVSV) and LV ejection fraction (LVEF) were calculated using

169   Simpson's biplane method utilizing both apical four and two chamber orientations.

170

171   *Dynamic LV function.* For the LV longitudinal ε-volume loops an apical four chamber view was

172   used to assess simultaneous LV longitudinal ε and volume. Myocardial ε and volume were

173   assessed offline using the apical 4 chamber view and dedicated software (EchoPac V113.05,

174   GE Healthcare, Horton, Norway). A region of interest (ROI) was placed from the basal septum

175   to the basal lateral wall of the LV enclosing the myocardium. The ROI was divided in six

176   myocardial segments, providing segmental and global longitudinal ε. Global longitudinal ε was

177   used for subsequent analysis.

178

179 Temporal global longitudinal ε values were exported to a spreadsheet (Excel, Microsoft Corp,

180 Washington, US). Global temporal longitudinal ε values were divided in 300 ε values for systole

181 and 300 ε values for diastole by cubic spline interpolation. For both systole and diastole, these

182 300 ε values were split into 5% increments of the cardiac cycle providing longitudinal ε values

183 at 11 time points in systole and 11 time points in diastole. Concomitant time points for the ε

184 values were used in the same image and cardiac cycle to trace monoplane LV volumes to

185 provide simultaneous ε and volume values. For each measurement phase a LV longitudinal ε-

186 volume loop was created for each participant after which a mean LV longitudinal ε-volume

187 loops for each phase was calculated.

188

189 Using the individual LV longitudinal ε-volume loops a linear regression line and a polynomial

190 of two orders were applied to both diastolic and systolic parts of the loop. This derived

191 polynomial equation allowed the derivation of ε-values per % increment of LVEDV (i.e. a value

192 for ε at 100% of LVEDV, at 99% of LVEDV etc.), within the working range of the heart. The

193 longitudinal ε-volume relationship was assessed by *1)* linear slope of systolic ε-volume relation

194 during the first 5% of volume change (ESslope), *2)* linear slope of ε-volume relation during

195 systole (Sslope), *3)* End-systolic peak ε (peak ε), *4)* Diastolic uncoupling (i.e. difference systolic

196 vs diastolic ε at the same volume), during early filling (UNCOUP_ED), *5)* during atrial

197 contraction (UNCOUP_LD), *6)* during the entire cardiac cycle (UNCOUP) *7)* linear slope of

198 diastolic ε-volume relation during the first 5% of volume change (EDslope) and *8)* linear slope

199 of diastolic ε-volume relation during the last 5% of volume change (LDslope). Parameters 2 till

200 5 were collected as previously described.(10) Parameter 1, 7 and 8 were calculated as the

201 gradient of the linear regression line, over the by polynomial equation derived ε-values and

202 matching volumes for the first or last 5% of volume change within the working range of the

203 heart, providing insight in the contribution of early and late contraction and or relaxation to

204 volume change. Parameter 6 was calculated as the mean difference between all values for

205 systolic and diastolic ε contribution at a certain % of LVEDV within the working range of the

206 heart, presenting a normalized estimation of the area between the curves (Figure 1, adapted

207 from(10)). Previously collected data on the intra-user variability of the loop characteristics

208 presented good to excellent (0.737-0.950) intraclass correlations for our loop

209 characteristics.(10)

210

211 *Statistical analysis*

212 Data for each time point is expressed as mean ± standard deviation. Normality of data

213 distribution was examined using a Kolmogorov-Smirnov test. In case of non-Gaussian

214 distribution, log-transformation was applied after which the data were re-examined. A two-

215 way repeated measures ANOVA (IBM SPSS statistics version 23) was used to assess the effect

216 of an afterload increase on the LV longitudinal ε-volume loop (time: baseline *versus* anti-G suit

217 *versus* recovery) and whether this effect differed between young and older participants

218 (time*age). In case of significant differences across time, a Bonferonni Post Hoc analysis was

219 applied to establish differences between pairwise time point comparisons. To provide further

220 insight in the temporal changes within the systolic or diastolic ε-volume relation in response to

221 inflation of the anti-G suit, the contribution of ε to volume change during both systole and

222 diastole was assessed by comparison of the ε-values at 10% increments of EDV.

223

224

225 **RESULTS**

226 **Study population**

227 Five out of the 42 recruited patients were excluded prior to the measurements due to

228 hypertension (n=2), atrial fibrillation (n=2), presence of an old undiagnosed myocardial

229   infarction (n=1) observed during medical screening. One participant failed to complete the

230   testing session and one participant did not attend, resulting in 35 participants who completed

231   all procedures, consisting of 19 young and 16 older participants.

232

233   **Aim 1: Acute increase in LV afterload**

234   *Central haemodynamics (Nexfin).* Immediately after inflation of the anti-G suit an increase in

235   HR, DBP, MAP and SVR was observed (all $P<0.05$, Table 2). No changes in SBP and dP/dT

236   were present (Table 2). Central haemodynamics returned to baseline upon recovery (all $P>0.05$,

237   Table 2).

238   *LV volumes and cardiac output.* A small, but significant decrease was present in LVEDV,

239   LVSV and LVEF (all $P<0.05$) immediately after inflation of the anti-G suit, while no changes

240   in LVESV were observed (Table 3). LV characteristics returned to baseline during recovery

241   (all $P>0.05$, Table 3).

242   *Dynamic LV function (ε-volume loop).* A marked change in the shape of the LV longitudinal ε-

243   volume loop was present after inflation of the anti-G suit (Figure 2). A significant decrease in

244   peak ε and EDslope was present, whilst a significant increase in UNCOUP_ED, UNCOUP_LD,

245   UNCOUP and LDslope was observed (all $P<0.05$). No changes in Sslope and ESslope were

246   present. (Table 3). All LV longitudinal ε-volume loop parameters returned to baseline during

247   recovery (all $P>0.05$, Table 3). Inflation of the anti-G suit did not change systolic ε contribution

248   to volume change at 90, 80, 70, 60 and 50% of EDV (all $P>0.05$), whilst a significant decrease

249   was found in diastolic ε contribution to volume change at 90, 80, 70 and 60% of EDV after

250   inflation of the Anti-G suit (all $P<0.05$, Table 4).

251

252   **Aim 2: Effects of age on acute increase in afterload**

253 *Central haemodynamics (Nexfin).* At baseline, older participant presented with higher SBP,

254 DBP, MAP and SVR (Table 2). Acute manipulation of the afterload induced comparable

255 changes in central haemodynamics compared to young subjects, except for a significantly larger

256 increase in DBP in older participants compared to younger participants (Table 2).

257 *LV volumes and cardiac output.* No significant differences in echocardiographic measures of

258 LV structure or function were present at baseline (Table 3). Older participants showed a

259 comparable decrease in measures of LV volumes and cardiac output in response to inflation of

260 the anti-G-suit compared to their younger peers (Table 3, Figure 3).

261 *Dynamic LV function (ε-volume loop).* At baseline, older participants presented less coherence

262 between the systolic and diastolic ε-volume relation, and showed significantly higher values for

263 UNCOUP_ED, UNCOUP_LD and UNCOUP as well as LDslope (Table 3). Older subjects also

264 demonstrated a smaller change in diastolic ε contribution to volume change at 90, 80, 70, 60

265 and 50% of EDV compared to young subjects (Table 4). No significant differences were found

266 between young and older subjects in the changes in dynamic LV function upon inflation of the

267 anti-G suit (Table 3+4, figure 4A and 4B).

268

269

270 **DISCUSSION**

271 We present the following novel findings; (1) An acute increase in LV afterload resulted in

272 uncoupling between the systolic and diastolic parts LV longitudinal ε-volume loop, (2) despite

273 a decrease in peak ε, no further changes in the systolic ε-volume relationship were observed,

274 while a shift of the diastolic ε-volume relationship was present (for any given volume, the

275 longitudinal length is shorter during diastole than in systole), highlighting the importance of

276 examining diastolic characteristics, (3) older participants presented with greater uncoupling of

277 the systolic and diastolic ε-volume relation at baseline compared to their younger peers, whilst

278   no differences were found between groups for any of the traditional measures for LV volumes

279   and cardiac output, and (4) acute manipulation of LV afterload in older men resulted in similar

280   effects on the LV longitudinal ε-volume loop compared to young healthy males. Taken together,

281   these data indicate that an acute increase in LV afterload leads to changes in diastolic LV

282   function that can be detected using the LV longitudinal ε-volume loop, with younger and older

283   humans demonstrating similar changes in LV longitudinal ε-volume loop characteristics in

284   response to acute increases in LV afterload. This highlights the potency of the LV longitudinal

285   ε-volume loop in providing additional information on the effect of (patho)physiological stimuli

286   on dynamic LV function.

287

288   *Effects of anti-G suit inflation on the systolic ε-volume relation*

289   After inflation of the anti-G suit we observed an increase in MAP and SVR, findings which can

290   be expected in the presence of an increase in afterload. In line with the findings of Donal *et*

291   *al*.(4), who reported a small decrease in peak ε with an acute increase in LV afterload, we

292   confirmed the presence of a small decrease in peak ε after inflation of the Anti-G suit. Despite

293   the reduction in peak ε, we found no change in the magnitude of LV longitudinal shortening

294   per 10% increment of LV volume change (Table 4). This indicates that an immediate increase

295   in LV afterload does not importantly change the temporal relation between LV volume and

296   longitudinal ε during systole.

297

298   *Effects of anti-G suit inflation on the diastolic ε-volume relation*

299   In contrast to the systolic phase, the diastolic phase of the ε-volume loop presented marked

300   changes in response to changes in central haemodynamics. First, under unloaded conditions,

301   early diastolic rapid LV relaxation creates an atrioventricular pressure gradient across the mitral

302   valve, causing the LV to suck blood from the left atrium (LA) to the LV (i.e. early filling).(9)

303   During early filling, LV longitudinal strain relaxation dominates volume displacement, leading

304   to a relatively large decrease in strain for a given decline in LV volume. After the early filling,

305   when the atrioventricular pressure gradient approaches 0 mmHg, the LA contracts to increase

306   atrial pressure and push blood from the LA to the LV (i.e. atrial filling). During this phase,

307   volume displacement by atrial contraction dominates LV longitudinal relaxation. Consequently,

308   the diastolic relation between LV longitudinal ε-volume crosses the systolic relation between

309   LV longitudinal ε-volume, as presented in Figure 2. Our study adds the novel observation that

310   inflation of the Anti-G-suit caused a rightward shift of the diastolic ε-volume relation. This shift

311   suggests a more dominant role for atrial volume displacement compared to LV longitudinal

312   relaxation during LV filling. Indeed, an attenuated EDslope and smaller change in longitudinal

313   ε value across a 10% increment of EDV (Table 4) confirms the reduced contribution of LV

314   longitudinal deformation to LV filling upon an increase in LV afterload, which may explain the

315   observed slightly reduced LVEDV. A possible explanation for these characteristic changes in

316   the diastolic relation between LV longitudinal ε-volume during elevation in afterload is an

317   alteration or delay in LV untwist.(2) Dong *et al.* showed that LV torsion is impaired under the

318   influence of an increase in afterload.(5) Impairment of LV torsion results in less twist and

319   energy storage in the myocardial fibers during the (systolic) ejection phase and, consequently,

320   less energy release during early diastole (i.e. recoil) to facilitate LV suction and thus filling.(2,

321   21) Taken together, our results indicate that an increase in afterload leads to a characteristic

322   shift in the LV longitudinal ε-volume loop, especially during diastole, that may contribute to

323   less suction and less effective filling of the ventricle. Assessing LV longitudinal ε-volume loop

324   characteristics alongside other ventricular mechanics could potentially provide complemental

325   data and should be subject of future studies.

326

327   *Effects of age*

328   Ageing is related to an increase in arterial stiffness causing an increase in systemic blood

329   pressure and LV afterload.(3) In line with this we observed higher blood pressure and SVR at

330   baseline in our older participants compared to younger participants. We did not observe any

331   age-related differences in the characteristics of the systolic phase of the LV longitudinal ε-

332   volume loop or contribution of ε to volume change (Table 4). However, young and older

333   subjects demonstrate differences in the diastolic phase of the LV longitudinal ε-volume loop.

334   Specifically, older participants showed a rightward shift of the diastolic ε-volume relation. This

335   rightward shift may reflect an age-related reduction in ventricular compliance, caused by an

336   increase in LV stiffness. Using invasive cardiac catheterisation Fujimoto *et al.*(7) observed

337   preserved systolic LV function in a healthy ageing population, but steepening of the diastolic

338   pressure–volume relationship in healthy older participants (i.e. reduction in ventricular

339   compliance). These findings support the presence of age-related differences in the LV

340   longitudinal ε-volume loop, with specific changes in diastolic LV function.

341

342   After inflation of the Anti-G suit, both young and older participants showed a similar increase

343   in uncoupling with comparable declines in LVEDV and SV. This indicates that, although the

344   longitudinal structure-function relationship in older participants is altered under resting

345   conditions, an increase in LV afterload leads to a characteristic change in dynamic cardiac

346   function, especially visible during diastole. This similarity in responsiveness between young

347   and older participants may partially be explained by the inclusion of healthy older individuals,

348   who demonstrated no structural or functional impairment of the heart. Our findings cannot be

349   simply extrapolated to subjects with impaired cardiac function and/or presence of

350   cardiovascular disease.

351

352   *Limitations.* Due to the nature of our intervention, we were not able to perform a full

echocardiographic assessment at all time points. We chose to collect images that were important for our ε-volume loop analysis. As a result, we were unable to determine all standard diastolic echocardiographic parameters, including twist or torsion parameters. In addition, current ε-volume loop analysis has been performed on 2D-4CH images, using monoplane derived volumes. Due to temporal differences between the 4CH and 2CH images, bi-plane volumes cannot be derived for this purpose. Optimizing current techniques through Tri-plane or 3D-imaging is warranted. Another limitation is that besides an increase in afterload the anti-G suit also affects preload and cardiac contractility(16) in order to adapt to the inflated anti-G garments and increased afterload. We indeed observed a slight decrease in LVEDV, suggesting presence of a decline in preload. The effects of a reduction in preload on the ε-volume loop have been previously examined by Schneider *et al*., who observed a decrease in peak ε, but no changes in the shape of the LV longitudinal ε-volume loop.(20) This means that the changes in the LV longitudinal ε-volume loop in our study can be primarily attributed to the alterations in LV afterload. Finally, in current study an normalized estimation of the area between the curves was utilized to measure the coherence between the systolic and diastolic phase of the LV strain-volume loop. Automatizing the current method should allow for calculating a more global area between the curve value and should be subject of future studies.

In conclusion, our findings indicate that an acute increase in LV afterload may lead to immediate and afterload-dependent changes within the diastolic phase of the LV longitudinal ε-volume loop, and thus less coherence between systolic and diastolic phase of the LV longitudinal ε-volume loop. Although older participants demonstrated an age-related decline in longitudinal relaxation at rest, an acute increase in LV afterload results in similar changes in coherence of the LV longitudinal ε-volume loop characteristics as in younger participants. The potential clinical implication of our observations is that the LV longitudinal ε-volume loop may

378   provide novel insight into dynamic cardiac function, which may help to understand and explain

379   the impact of (patho)physiological processes on the heart *in vivo* in humans.

380

381   *Perspectives and Significance*

382   With this study we provide novel insights into our understanding of the influence of

383   haemodynamical changes on systolic and diastolic cardiac function by demonstrating that

384   changes in afterload lead to suppression of diastolic relaxation. The ability of our (novel)

385   technique to simultaneously assess systolic and diastolic ventricular function in a non-invasive

386   way provides major advantages over current techniques and provide a novel quick method to

387   assess overall ventricular function in the near future.

388

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472 **Figure 1** - **A schematic view of the methods used to assess the ε-volume loops.** The black

473 line represents the ε-volume loop, the thick part represents the systolic phase and the thin line

474 the diastolic phase. We assessed the ε-volume loop by a) ε-volume relation at the first 5% of

475 systole (i.e. ESslope; red dotted line), b) ε-volume relation across the systolic phase (i.e. Sslope,

476 orange dashed line), c) peak ε at end-systole (i.e. peak ε, purple arrow), d) difference in systolic

477 vs diastolic ε during early filling (i.e. UNCOUP_ED, pink arrow), e) difference in systolic vs

478 diastolic ε during atrial contraction (i.e. UNCOUP_LD, dark blue arrow), f) difference in

479 systolic vs diastolic ε during the entire cardiac cycle (ie UNCOUP, light blue area), g) ε-volume

480 relation at the first 5% of diastole (i.e. EDslope, yellow dashed line) and h) ε-volume relation

481 at the last 5% of diastole (i.e. ESslope, green dashed line).

482

483 **Figure 2 – Mean ε-volume loops prior to and after acute manipulation of afterload.** Data

484 represents mean longitudinal ε-volume loops over the entire study population (n=35) at baseline

485 (black lines) and after an increase in afterload (filled grey lines). The thick lines represent the

486 systolic ε-volume relation, whilst the thin lines represent the diastolic ε-volume relation.

487

488 **Figure 3 – Overview of the influence of an acute afterload manipulation and age on the**

489 **most important haemodynamic, functional and ε-volume loop parameters.** Data represents

490 the mean ± SEM values for the measurements of LV haemodynamics (i.e. MAP),   ε-volume

491 loop characteristics (i.e. UNCOUP) and LV function (i.e. LVEF) at baseline, after acute

492 manipulation of afterload and after a recovery period. The white bars represent young

493 participants while the black bars represent older participants.

494

495 **Figure 4 - Mean ε-volume loops prior to and after acute manipulation of afterload in**

496 **young and older participants.** Data represents mean longitudinal ε-volume loops in A) young

497   participants (24±2 years of age, n=19) and B) older participants (67±5 years of age, n=16). The

498   solid black lines represent the ε-volume loop at baseline, whilst the grey lines represent the ε-

499   volume loop after an increase in afterload. The thick lines represent the systolic ε-volume

500   relation, whilst the thin lines represent the diastolic ε-volume relation.

501

502 **Table 1 – Population Characteristics**

Characteristics	Young	Older	P-value
Height (cm)	182±6	180±6	0.28
Weight (kg)	82±12	85±11	0.46
SBP (mmHg)	132±13	144±13	**0.01**
DBP (mmHg)	80±8	85±6	**0.04**
HR (BPM)	68±13	70±12	0.66

503   Baseline group characteristics as measured manually during the screening procedure.

504   SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; HR=Heart Rate.

505 **Table 2 –** Data represents the mean±SD of central haemodynamic measurements at baseline, after an increase of the afterload and after an recovery

506 period in a group of young participants and older participants.

Characteristics		Baseline	Anti-G suit	Recovery	Intervention	Group	Intervention*Group
Central haemodynamics (n=33)							
HR (bpm)	*Young*	62±14	66±17	62±14	**<0.01***	0.80	0.55
	Older	62±10	65±11	60±9			
SBP (mmHg)	*Young*	133±16	138±16	133±15	0.08	**0.02**	0.69
	Older	146±30	157±25	150±22			
DBP (mmHg)	*Young*	73±9	81±11	74±9	**<0.01***	**<0.01**	**0.02**
	Older	80±13	99±13	85±10			
MAP (mmHg)	*Young*	93±12	99±12	94±12	**<0.01***	**<0.01**	0.07
	Older	105±16	121±15	109±12			
SVR (dyn•s/cm^5)	*Young*	1026±225	1123±192	1055±239	**0.04**	**<0.01**	0.22
	Older	1907±788	2366±914	2145±645			
dP/dT (mmHg/s)	*Young*	1187±262	1158±287	1210±284	0.72	0.98	0.22
	Older	1216±710	1263±791	1117±616			

507 Symbols denote P<0.05 between ANTI-G-Suit vs. Baseline and Recovery=*, all 3 Phases=[†] and Baseline vs. ANTI-G-Suit=[‡]. HR=Heart Rate;
508 SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; MAP=Mean Arterial Pressure; SVR=Systemic Vascular Resistance; dP/dT=Delta
509 Pressure/Delta Time;
510

511 **Table 3** – Data represents the mean±SD of echocardiographic measurements at baseline, after an increase of the afterload and after an recovery
512 period in a group of young participants and older participants.

Dynamic *LV function (ε-volume loop)*		Baseline	Anti-G suit	Recovery	Intervention	Group	Intervention*Group
ESslope (%/ml)	Young	0.31±0.12	0.31±0.11	0.31±0.16	0.40	0.59	0.44
	Older	0.37±0.15	0.31±0.16	0.31±0.17			
Sslope (%/ml)	Young	0.35±0.08	0.34±0.07	0.36±0.09	0.24	0.18	0.59
	Older	0.40±0.11	0.38±0.09	0.39±0.16			
Peak ε (%)	Young	-20.3±2.4	-19.0±2.2	-20.3±2.6	**<0.01[†]**	0.18	0.06
	Older	-22.0±2.2	-19.9±2.2	-20.9±2.8			
UNCOUP_ED (AU)	Young	-1.32±1.38	0.68±1.54	-0.45±1.79	**<0.01***	**<0.01**	0.68
	Older	-0.23±1.42	2.31±1.35	0.55±1.69			
UNCOUP_LD (AU)	Young	-0.32±1.48	1.09±1.65	0.66±1.73	**<0.01[‡]**	**<0.01**	0.34
	Older	0.91±1.75	2.64±1.34	1.20±1.32			
UNCOUP (AU)	Young	-0.90±1.13	0.82±1.53	-0.08±1.70	**<0.01***	**<0.01**	0.32
	Older	0.15±1.45	2.51±1.44	0.56±1.15			
EDslope (%/ml)	Young	0.50±0.14	0.30±0.11	0.47±0.20	**<0.01***	0.12	0.68
	Older	0.43±0.15	0.21±0.10	0.43±0.23			
LDslope (%/ml)	Young	0.15±0.09	0.33±0.12	0.19±0.16	**<0.01***	**<0.01**	0.54
	Older	0.31±0.13	0.47±0.17	0.29±0.12			
LV volumes and cardiac output (n=30)							
LVEDV (ml)	Young	113±18	107±12	109±14	**0.02**	0.15	0.70
	Older	102±15	96±23	101±24			
LVESV (ml)	Young	47±9	47±7	47±8	0.81	0.26	0.70
	Older	43±13	42±11	44±13			
LVSV (ml)	Young	65±12	60±7	63±7	**<0.01***	0.11	0.74
	Older	58±14	54±14	58±12			
LVEF (%)	Young	58±4	56±4	57±4	**0.05[‡]**	0.98	0.84
	Older	58±4	56±4	57±4			

513 Symbols denote P<0.05 between ANTI-G-Suit vs. Baseline and Recovery=*, all 3 Phases=[†] and Baseline vs. ANTI-G-Suit=[‡]. LVEDV= Left
514 Ventricular End Diastolic Volume; LVESV=Left Ventricular End Systolic Volume; LVEF=Left Ventricular Ejection Fraction

2

515 **Table 4** – ε-values per 10% increment in volume during systole and diastole
516

Characteristics		Baseline	Anti-G-suit	Recovery	Intervention	Group	Intervention*Group
Dynamic LV function (n=35)							
Systolic ε at 90% EDV	*Young*	-3.58±1.29	-3.67±1.12	-3.31±1.71	0.50	0.64	0.77
	Older	-3.67±1.47	-3.30±1.12	-3.18±1.59			
Systolic ε at 80% EDV	*Young*	-7.29±1.70	-7.34±1.36	-6.99±2.15	0.33	0.88	0.52
	Older	-7.93±2.24	-6.96±1.86	-6.91±2.31			
Systolic ε at 70% EDV	*Young*	-11.19±1.92	-11.18±1.45	-10.98±2.39	0.26	0.54	0.38
	Older	-12.30±2.72	-10.94±2.23	-10.99±2.66			
Systolic ε at 60% EDV	*Young*	-15.28±2.02	15.21±1.70	-15.23±2.52	0.24	0.29	0.30
	Older	-16.77±2.89	-15.24±2.30	-15.45±2.75			
Systolic ε at 50% EDV	*Young*	-19.56±2.29	-19.42±2.50	-19.78±2.89	0.31	0.18	0.36
	Older	-21.35±2.90	-19.86±2.32	-20.27±2.95			
Diastolic ε at 90% EDV	*Young*	-3.17±1.29	-4.88±1.65	-3.74±1.89	**<0.01***	**<0.01**	0.70
	Older	-4.42±1.35	-6.17±1.42	-4.51±1.41			
Diastolic ε at 80% EDV	*Young*	-5.98±1.55	-8.55±2.00	-6.69±2.52	**<0.01***	**<0.01**	0.57
	Older	-8.22±1.68	-10.44±1.89	-8.04±1.61			
Diastolic ε at 70% EDV	*Young*	-9.63±1.84	-12.17±2.04	-10.29±2.73	**<0.01***	**<0.01**	0.42
	Older	-12.30±1.90	-14.09±2.18	-11.79±1.91			
Diastolic ε at 60% EDV	*Young*	-14.12±2.36	-15.73±1.81	-14.55±2.74	**0.02***	**<0.01**	0.32
	Older	-16.64±2.16	-17.14±2.27	-15.76±2.40			
Diastolic ε at 50% EDV	*Young*	-19.46±3.35	-19.23±1.65	-19.46±3.11	0.24	**<0.01**	0.37
	Older	-21.25±2.83	-19.58±2.36	-19.94±3.27			

517 Symbols denote P<0.05 between ANTI-G-Suit vs. Baseline and Recovery=*. EDV=End diastolic volume
518