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1 **Direct cardiac versus systemic effects of inorganic nitrite on human left ventricular**
2 **function**

3
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14 clinical responsibility for patients.

15
16 **Short Title:** Effect of inorganic nitrite on LV function

17
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27 from the corresponding author upon reasonable request.

28

29

30 **ABSTRACT**

31 **Background**

32 Inorganic nitrite is a source of nitric oxide (NO) and is considered a potential therapy in
33 settings where endogenous NO bioactivity is reduced and left ventricular (LV) function
34 impaired. However, the effects of nitrite on human cardiac contractile function, and the
35 extent to which these are direct or indirect, are unclear.

36 **Methods and Results**

37 We studied 40 patients undergoing diagnostic cardiac catheterisation who had normal LV
38 systolic function and were not found to have obstructive coronary disease. They received
39 either an intracoronary sodium nitrite infusion (8.7-26 $\mu\text{mol}/\text{min}$, $n=20$) or an intravenous
40 sodium nitrite infusion (50 $\mu\text{g}/\text{kg}/\text{min}$, $n=20$). LV pressure-volume relations were recorded.
41 The primary end point was LV end-diastolic pressure (LVEDP). Secondary end points
42 included indices of LV systolic and diastolic function. Intracoronary nitrite infusion induced
43 a significant reduction in LVEDP, LV end-diastolic pressure-volume relationship (EDPVR)
44 and the time to LV end-systole (LVEST) but had no significant effect on LV systolic function
45 or systemic haemodynamics. Intravenous nitrite infusion induced greater effects, with
46 significant decreases in LVEDP, EDPVR, LVEST, LV $\text{dP}/\text{dt}_{\text{min}}$, tau, and mean arterial
47 pressure.

48 **Conclusions**

49 Inorganic nitrite has modest direct effects on human LV diastolic function, independent of
50 LV loading conditions and without affecting LV systolic properties. However, the systemic
51 administration of nitrite has larger effects on LV diastolic function which are related to
52 reduction in both preload and afterload. These contractile effects of inorganic nitrite may
53 indicate a favourable profile for conditions characterized by LV diastolic dysfunction.

54 **Keywords:** Inorganic nitrite, Nitric oxide, HFpEF, Diastolic function, Pressure-volume
55 relationship

56

57 **New and Noteworthy (max 75 words)**

58 This is the first study to assess the direct and indirect effects of inorganic nitrite on invasive
59 measures of left ventricular function in humans *in vivo*. Inorganic nitrite has a modest direct
60 myocardial effect, improving diastolic function. Systemic administration of nitrite has larger
61 effects related to alterations in cardiac preload and afterload. The changes induced by nitrite
62 appear favourable for potential use in conditions characterised by LV diastolic dysfunction.

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66

67 **Introduction**

68 Nitric oxide (NO) has important roles in the physiological regulation of cardiovascular
69 function while dysfunction of endogenous NO production or NO-cyclic GMP (cGMP)
70 signaling are implicated in the pathophysiology of several cardiovascular diseases[1, 2].
71 Accordingly, strategies to increase local tissue concentrations of NO or to enhance NO-
72 dependent signaling may have therapeutic potential. Inorganic nitrite (NO_2^-) is of interest in
73 this regard as it can be reduced to NO and have effects similar to NO donors but tolerance
74 does not develop to its effects with continued use, unlike the case with organic nitrates[3, 4].
75 Inorganic nitrite is a vasodilator, affecting both arterial[5-7] and venous tone[6]. In the
76 coronary bed, nitrite is relatively selective for conduit versus resistance vessels[8]. When
77 given via intravenous infusion, nitrite causes vasodilatation and a reduction in central blood
78 pressure[7]. Nitrite also inhibits platelet aggregation[9, 10] and can improve mitochondrial
79 efficiency[11]. Previous experimental and clinical studies have therefore explored the
80 potential therapeutic benefit of nitrite in conditions such as myocardial ischemia-
81 reperfusion[12] [13], pulmonary hypertension[14], cerebral vasospasm[15] and impaired
82 exercise capacity in heart failure[16, 17].

83 Endogenously generated NO induces direct acute effects on the onset of myocardial
84 relaxation and on diastolic distensibility, independent of changes in systolic function or
85 systemic loading. A selective NO- and cGMP/protein kinase G (PKG)-dependent earlier
86 onset of relaxation without change in systolic function was found in isolated mammalian
87 cardiomyocytes and isolated hearts[18, 19]. NO also reduced diastolic stiffness[19]. Similar
88 effects on onset of LV relaxation and LV diastolic distensibility were observed in human
89 subjects *in vivo* after acute intracoronary infusion of substance P to trigger the endogenous
90 release of NO[20]. Consistent with these effects, it has been suggested that dysfunction of
91 NO-cGMP signaling contributes to left ventricular (LV) diastolic dysfunction both

92 experimentally and in patients[21-23]. As such, the clinical utility of nitrite to enhance
93 relaxation and diastolic function is of interest. However, the direct myocardial effects of
94 nitrite in the human heart and their relationship to its systemic effects have not been
95 established. In this study, we investigated the effect of either intracoronary or intravenous
96 nitrite infusion on LV contractile function.

97

98 **Methods**

99 The data that support the findings of this study are available from the corresponding author
100 on reasonable request.

101

102 *Participants*

103 Invasive LV pressure-volume (PV) studies were performed on patients (n=40) with suspected
104 coronary artery disease who were referred for diagnostic coronary angiography. All subjects
105 were known to have normal left ventricular systolic function on echocardiography. Written
106 informed consent was obtained prior to cardiac catheterization and the research study was
107 performed at the end of the diagnostic procedure if there was an absence of significant
108 epicardial coronary artery disease (<50% stenosis on coronary angiography and/or a
109 fractional flow reserve >0.80). Patients were also excluded if they had heart failure (either a
110 pre-existing diagnosis or a current clinical syndrome consistent with heart failure), clinically
111 significant valve disease, or had a history of glucose-6-phosphate dehydrogenase deficiency.
112 Patients were required to be in sinus rhythm at the time of assessment, with atrial fibrillation
113 and ventricular bigeminy/trigeminy being considered excluding factors. The study complied
114 with the *Declaration of Helsinki* and was approved by the institutional ethics committee
115 (Reference: 12/LO/1067).

116

117 *Study protocols*

118 We studied either intracoronary nitrite infusion (n=20) or intravenous nitrite infusion (n=20).
119 Radial or femoral arterial access was used for diagnostic coronary angiography at the
120 discretion of the operator. A second arterial puncture was required for patients receiving
121 intracoronary infusion. All patients received unfractionated heparin (5000 IU bolus), with
122 additional doses as required to maintain an activated clotting time (ACT) of >250 s.

123 For intracoronary infusion studies, a 6Fr guide catheter was positioned at the ostium
124 of the left main coronary artery. Patients first received a normal saline infusion for at least 5
125 minutes during which stable LV function parameters and BP were confirmed. They next
126 received an intracoronary infusion of sodium nitrite (NaNO_2 , Tayside NHS, UK) at 8.7
127 $\mu\text{mol}/\text{min}$ for 5 min, followed by 26 $\mu\text{mol}/\text{min}$ for 5 min. The higher dose is estimated to
128 achieve a maximal intracoronary concentration of approximately 1000 μM , using average
129 resting coronary blood flow estimates as described previously[8] and is equivalent to
130 concentrations that when administered intra-arterially in the peripheral circulation are locally
131 active (i.e. devoid of systemic effects)[7]. For intravenous infusion studies, sodium nitrite
132 was administered at 50 $\mu\text{g}/\text{kg}/\text{min}$ for 7 min via a canula in a large antecubital fossa vein.
133 This dose was chosen to achieve physiologically significant reduction in systemic blood
134 pressure and pulmonary capillary wedge pressure (i.e. both afterload and preload)[16]. The
135 local concentration of nitrite in the coronary circulation after systemic infusion is estimated to
136 be >100-fold lower than after intra-coronary infusion but achieves significant reduction in
137 peripheral loading due to its generalized systemic actions. The direct myocardial actions of
138 intracoronary nitrite could therefore be compared with the indirect effects (due to altered
139 peripheral loading) of systemic nitrite. A micromanometer-conductance catheter (CD
140 Leycom, Netherlands) was placed in the left ventricle to record steady-state LV PV relations
141 via an Intra-Cardiac Analyser (INCA) console (CD Leycom, Netherlands). Recordings of PV

142 relations were made immediately prior to, and immediately after the nitrite infusion.
143 Measurements were also made of heart rate, blood pressure and the ECG. All patients had a
144 3D transthoracic echocardiogram to estimate LV volumes, which were used for volumetric
145 calibration. Dedicated software (CD Leycom Netherlands) was used for analysis of PV loop
146 data including LV systolic and diastolic indices, and ventricular-arterial coupling (VA
147 coupling, calculated as the ratio of arterial elastance to end systolic elastance, E_a/E_{es})[24,
148 25]. Recordings were made at end-expiration. Ten beats were averaged to provide each data
149 point.

150 We also quantified first-phase ejection fraction (EF1), which represents the proportion
151 of blood ejected from the LV from the onset of systole to the time of the first peak of LV
152 pressure. EF1 has been suggested as an index that assesses systolic function early during
153 contraction and reflects systolic-diastolic coupling[26, 27].

154

155 *Sample size and study end-points*

156 Previous work reported that a bi-coronary infusion of sodium nitroprusside induced a
157 decrease in LV end-diastolic pressure (LVEDP) from 18 ± 5 mmHg to 12 ± 3 mmHg[28],
158 equating to an effect size of 1.37. We estimated that a single left coronary infusion of sodium
159 nitrite might have an effect of two thirds of this magnitude, i.e. an effect size of 0.91.

160 Therefore, with an alpha of 0.05 and power (1-beta) of 0.95, the required sample size was 18
161 for a primary end-point of reduction in LVEDP. To allow an ~10% margin for patients who
162 may fail to complete the protocol (e.g. due to technical issues), 20 patients per group were
163 recruited. Exploratory secondary end-points included other measures of LV systolic and
164 diastolic function.

165

166 *Statistical analyses*

167 Analyses were performed using GraphPad Prism 8 (GraphPad Software Inc, USA). The
168 Shapiro-Wilk test was used to assess normality. Data are expressed as mean \pm SD for
169 parametric data and median [IQR] for non-parametric data. Intracoronary data were
170 compared by repeated measures ANOVA with Tukey's post-test for multiple comparisons (or
171 non-parametric equivalent). Student's t test was used to compare the intravenous data as well
172 as the effect of intracoronary versus intravenous nitrite on PV parameters (change from
173 baseline). Linear regression analysis was used to test for correlation between measures of LV
174 structure and changes in the primary end-point. $P < 0.05$ was considered statistically
175 significant.

176

177

178 **Results**

179 The baseline characteristics of the patients included in the study are shown in **Table 1**. Risk
180 factors for coronary artery disease such as hypertension, smoking, hypercholesterolemia and
181 diabetes were common but well matched between the intracoronary and intravenous infusion
182 groups. All patients had a normal LV ejection fraction (EF) on echocardiography. However,
183 some of the patients had an elevated LV mass index (LVMI) and left atrial volume index.

184 The studies were performed without clinical complications in any patient.

185

186 ***Effects of intracoronary nitrite infusion***

187 Intracoronary nitrite had no significant effect on heart rate or mean arterial blood pressure
188 (MAP), consistent with a lack of systemic effect (**Figure 1A-B**). Markers of LV systolic
189 function, namely LV end-systolic elastance (Ees), stroke work and dP/dt_{max} were unaltered by
190 intracoronary nitrite (**Figure 1E-G**). However, there was a significant decrease in the primary
191 end-point, LVEDP, following intracoronary nitrite ($P=0.004$) (**Figure 1I**). When considered

192 as change from baseline, the 26 $\mu\text{mol}/\text{min}$ nitrite dose decreased LVEDP by 1.9 mmHg [-3.3,
193 -0.5] (mean [95% CI]), $P=0.006$. Intracoronary nitrite also significantly decreased EDPVR,
194 while the time to LV end-systole (LVEST) was decreased by 11 ms [-19, -4] ($P=0.002$) at the
195 higher dose of nitrite (**Figure 1J-K**). There were no significant changes in dP/dt_{min} , tau or LV
196 volumes (**Figure 1C-D,H,L**). There was no significant change in VA coupling (E_a/E_{es} $0.6 \pm$
197 0.2 at baseline, 0.6 ± 0.2 following 26 $\mu\text{mol}/\text{min}$ nitrite).

198

199 *Effects of intravenous nitrite infusion*

200 Intravenous nitrite resulted in a significant decrease in MAP of 6.9 mmHg [-4.3, -9.5] (mean
201 [95% CI]), $P<0.001$, but had no effect on heart rate (**Figure 2A-B**). Consistent with a
202 reduction in afterload, the arterial elastance (E_a) decreased from 2.1 ± 0.7 to 1.9 ± 0.7
203 ($P=0.002$). There was no change in the total peripheral resistance: mean change -0.7 [-2.2,
204 $+0.7$] (mean [95% CI]), $P=0.3$. Intravenous nitrite also induced a significant reduction in
205 LVEDV (-8.3 ml [-15.4, -1.1] (mean [95% CI]), $P=0.03$), consistent with a decrease in
206 preload (**Figure 2C**). No changes were observed in E_{es} (regardless of whether the outlier
207 data point is included or not, see **Figure 2E**) or dP/dt_{max} while stroke work decreased
208 significantly: -829 centijoules (cJ) [-1327, -331] (mean [95% CI]), $P=0.003$ (**Figure 2E-G**).
209 Intravenous nitrite caused a significant reduction in LVEDP from a baseline of 10.6 mmHg
210 [4.7 – 15.3] (median [IQR]) to 5.2mmHg [2.9, 9.9], $P<0.001$ (**Figure 2I**). Intravenous nitrite
211 also resulted in significant decreases in EDPVR, LVEST, dP/dt_{min} and tau (**Figure 2H, J-L**).
212 There was no significant change in ventricular-arterial coupling (E_a/E_{es} from 0.6 ± 0.2 to 0.5
213 ± 0.3 , $P=0.06$).

214

215 *Comparison of intracoronary and intravenous nitrite*

216 Representative PV loops showing the effect of intracoronary and intravenous nitrite infusion
217 are shown in **Figure 3** and suggest that intravenous infusion had a larger effect. **Figure 4**
218 shows a quantitative comparison of the effects of intravenous and intracoronary (26
219 $\mu\text{mol}/\text{min}$ nitrite) infusion. There was no significant difference at baseline between the
220 groups in MAP (99.0 mmHg [89.7, 110.3] (median [IQR]) vs 93.0 mmHg [86.0, 107.0] for
221 intracoronary vs intravenous) or LVEDP (11.0 mmHg [8.1, 14.3] vs 10.6 mmHg [4.7, 15.3]
222 for intracoronary vs intravenous). Intravenous nitrite had significantly greater effects than
223 intracoronary nitrite on MAP (**Figure 4I**), LV end-systolic pressure (LVESP) (**Figure 4F**),
224 and tau (**Figure 4D**). While the mean decrease in LVEDP following intravenous nitrite was
225 numerically greater than after intracoronary infusion (**Figure 4E**), this was not statistically
226 significant.

227 Intracoronary nitrite had no significant effect on EF1 ($P=0.5$ by 1-way ANOVA)
228 (**Figure 5A**) but intravenous nitrite induced a marked increase in EF1 (**Figure 5B-C**). From a
229 baseline of $23.0\pm 2.1\%$, the EF1 post-nitrite was $34.2\pm 3.1\%$ - a relative increase of
230 approximately 50% as illustrated by the representative traces in **Figure 5D-E**.

231

232 *Association between baseline LV structure and function and the effect of nitrite*

233 To assess whether inter-individual variation in the response to nitrite might be related to
234 baseline cardiac structure, we determined the association between LVMI and the magnitude
235 of change in LVEDP but found no significant correlation either in the intracoronary or
236 intravenous nitrite groups (**Figure 6A-B**). We also assessed whether the magnitude of
237 reduction in LVEDP was related to baseline LV EDPVR. There was a significant association
238 between the nitrite-induced decrease in LVEDP and baseline EDPVR for both the
239 intracoronary group ($R^2=0.33$, $P=0.008$) (**Figure 6C**) and the intravenous group ($R^2= 0.38$,
240 $P=0.004$) (**Figure 6D**). It should be noted, however, that when the data are analyzed as

241 percentage rather than absolute change in LVEDP, the association with baseline EDPVR is
242 no longer significant in the intracoronary group but remains significant in the intravenous
243 group.

244

245 **Discussion**

246 In this study, we have examined in detail the direct and indirect acute effects of inorganic
247 nitrite on contractile function of the human heart in patients undergoing diagnostic cardiac
248 catheterization who did not have coronary disease or heart failure. We demonstrate several
249 important findings that may have relevance to the potential therapeutic use of nitrite.

250 Firstly, inorganic nitrite delivered via the intracoronary route induces a small but
251 significant decrease in LVEDP and EDPVR and hastens the onset of LV relaxation (i.e.
252 reduces LVEST). These effects are not accompanied by any change in blood pressure or heart
253 rate, consistent with a local action on the heart. They occur without any alteration in indices
254 of LV systolic function, indicating a selective effect on diastolic properties of the heart. No
255 change in tau or LV dp/dt_{min} is observed following intracoronary nitrite, suggesting that
256 while nitrite improves LV distensibility (a passive property), there is a lack of effect on active
257 (ATP-dependent) myocardial relaxation. This pattern of effect on LV contractile function is
258 entirely consistent with prior studies reporting similar direct myocardial effects of NO donors
259 and NO-cGMP signaling on the onset of relaxation and diastolic stiffness, both in isolated
260 preparations and in humans in vivo[18, 20, 28, 29]. At a mechanistic level, such actions are
261 considered to involve cGMP/protein kinase G (PKG)-mediated phosphorylation of troponin I
262 and titin in cardiomyocytes[19, 30-32], although other NO-mediated mechanisms such as
263 altered S-nitrosylation of proteins[33, 34] or an effect on sympathetic nerve activity[35, 36]
264 may have a role. Taken together, the data from the intracoronary infusion study suggest that
265 inorganic nitrite has a direct and selective action on the myocardium to reduce ventricular

266 stiffness and hasten the onset of relaxation. However, the magnitude of these changes is small
267 in patients with normal LV function .

268 Secondly, the results of the intravenous infusion studies indicate that systemic
269 administration of nitrite significantly reduces LV preload (LVEDV) and afterload (MAP and
270 Ea) and is associated with more marked effects on LV diastolic function than observed after
271 intracoronary infusion. In addition to decreases in LVEDP, EDPVR and LVEST, intravenous
272 nitrite significantly accelerates tau and reduces LV dP/dtmin. The effects of intravenous
273 nitrite on LV function are likely to be due to the reduced loading of the heart rather than a
274 direct myocardial action since the local intracoronary concentration of nitrite achieved with
275 systemic infusion is estimated to be substantially lower than with intracoronary infusion (*see*
276 *Methods*). Therefore, intravenous nitrite-induced effects on LV diastolic function appear to
277 be driven mainly by its actions to reduce afterload and preload rather than a direct myocardial
278 action in this patient group. The effect of nitrite to reduce afterload and preload is well
279 established and the underlying mechanism is NO-mediated direct (endothelium-independent)
280 vasodilation[5-7].

281 Thirdly, intravenous nitrite infusion significantly increases EF1 - a hemodynamic
282 index that describes the proportion of LV ejection that occurs up to the time of maximal rate
283 of ventricular contraction[26]. EF1 has been proposed to reflect coupling between systolic
284 and diastolic function and therefore to provide a more integrated readout of overall changes
285 in cardiac function. EF1 may be reduced even in patients in whom the overall EF is within
286 the normal range, in which group it strongly correlates with abnormal LV diastolic function
287 as indexed by an elevated E/e' ratio (indicating elevated filling pressures) on
288 echocardiography[26]. Furthermore, a reduced EF1 may predict a worse prognosis in patients
289 with aortic stenosis or heart failure[27]. In the present study, the intravenous nitrite-induced
290 increases in EF1 suggest that an improvement in early ejection phase LV systolic function is

291 induced in addition to the enhancement of diastolic function. Given that no change in EF1
292 was observed with intracoronary infusion, it is likely that the increase following intravenous
293 nitrite infusion is due to the improved cardiac loading conditions rather than a direct
294 myocardial effect. This is consistent with prior data that nitrite decreases pressure wave
295 reflections in the arterial tree and decreases late systolic load on the LV[37] and that EF1 is
296 highly sensitive to changes in late systolic load[26]. The results with EF1 therefore further
297 emphasise that the predominant effects of intravenous nitrite appear to relate to its actions to
298 reduce afterload and preload.

299 A careful assessment of the systemic versus direct myocardial actions of either NO
300 donors such as sodium nitroprusside or of inorganic nitrite on cardiac contractile function has
301 not previously been undertaken. It was reported that intracoronary infusion of the NO donor
302 sodium nitroprusside reduced LVEDP and LVEST (similar to the current study) in patients
303 with normal LV function but that investigation did not involve measurement of PV relations
304 nor the assessment of the effects of systemic administration[28]. Recently, there has been
305 considerable interest in the therapeutic potential of nitrite in conditions associated with
306 decreased NO bioavailability, including heart failure[4]. The current study was performed in
307 patients with normal LV function in order to first establish the effects in this group but future
308 studies need to also study its effects in patients with impaired LV function – where the
309 magnitude or pattern of effect could be different. For example, the effects of the nitrite-NO
310 pathway are reportedly greater under hypoxic or ischemic conditions[6]. Extending the
311 findings on the acute effects of nitrite to its potential therapeutic value also requires
312 consideration of chronic administration. Nitrite can be administered in the form of oral nitrite
313 salts (such as sodium nitrite) or plasma nitrite levels can be elevated via inorganic nitrate, for
314 example an oral nitrate salt or by ingestion of beetroot juice. Previous studies in which oral
315 sodium nitrite (or nitrate) was administered chronically achieved post-dose plasma nitrite

316 concentration of a similar order of magnitude to those obtained after intravenous
317 administration, while trough levels remain increased compared to placebo[38, 39]. The
318 chronic administration of beetroot juice is able to achieve slightly lower levels of plasma
319 nitrite[40]. Future studies to assess the effect of chronic elevation of plasma nitrite on cardiac
320 function will be of value.

321 The pattern of effect of nitrite on LV contractile function raises the possibility that it
322 could be of value in heart failure. Both Heart Failure with Reduced Ejection Fraction
323 (HFrEF) and Heart Failure with Preserved Ejection Fraction (HFpEF) are characterized by
324 significant LV diastolic dysfunction. In HFrEF, it is already well established that a reduction
325 in the loading of the heart is beneficial and previous clinical trials in selected patient groups
326 showed benefit from a combination of organic nitrates and hydralazine[41]. HFpEF might
327 theoretically be especially amenable to nitrite therapy as there is quite good evidence of
328 impaired NO/cGMP signaling and abnormal loading in this condition[42, 43]. Furthermore,
329 acute nitrite administration is reported to reduce pulmonary capillary wedge pressure
330 (PCWP) during exercise in patients with HFpEF[16]. However, a recent randomised trial of
331 inhaled nitrite in HFpEF failed to show benefit with respect to echocardiographic filling
332 pressures or exercise capacity[17], although inhaled nitrite may be considered more
333 analogous to local than systemic delivery. A randomised trial studying the effects of 6 weeks
334 administration of an organic nitrate, isosorbide mononitrate, in patients with HFpEF also
335 failed to show benefit[44]. Whether chronic administration of inorganic nitrite (or dietary
336 manipulation to elevate nitrite levels) has different effects in this patient group merits further
337 study.

338

339 *Study limitations*

340 We studied a relatively small number of subjects, many of whom had risk factors for
341 cardiovascular disease and were on medications. Some of the patients also had increased LV
342 mass or raised LV filling pressures at baseline. As such, the study population is not
343 comparable to healthy subjects. The possibility that the effects of nitrite may vary depending
344 on risk factor cannot be excluded. The intracoronary group had a higher median LVMI than
345 the intravenous group but the values were within the normal range in both groups. We
346 looked for any correlation between LV mass and the magnitude of effect on LVEDP but
347 found no significant relationship. The study design did not include a placebo group but all
348 patients received an initial saline infusion (placebo) during which period the stability of LV
349 function parameters was confirmed. It was not practical for logistic reasons to randomise
350 allocation to intracoronary or intravenous nitrite since the two procedures were technically
351 different. Due to ethical and logistical considerations, it was also not feasible to perform both
352 intracoronary and intravenous nitrite in the same patients as doing this would have made the
353 study prohibitively long. The current study only looked at the acute effects of a single nitrite
354 infusion at rest. It is possible that the effects may be larger upon exercise and the results also
355 cannot necessarily be extrapolated to the effects of chronic administration. It is not known
356 how much bioactive NO is released within the myocardium from a given dose of nitrite in
357 this study and therefore the results are not generalisable to other NO donor drugs. This could
358 be addressed in future studies by assessment of blood plasma sampled from both the coronary
359 artery and the coronary sinus. EDPVR was assessed using the single beat method, rather than
360 through vena cava occlusion to induce a loading change, but the single beat method is known
361 to reliably detect the acute effects of interventions. As common in such clinical studies, our
362 dataset contained a number of outlier data points. However, repeating the analyses without
363 inclusion of such outliers did not materially alter the overall pattern of results.

364

365 *Conclusion*

366 We have undertaken a comprehensive characterisation of the acute effects of intracoronary
367 and intravenous nitrite on human cardiac contractile function, using doses designed to
368 achieve solely local myocardial or solely peripheral vascular effects, respectively. Our
369 findings demonstrate that nitrite has modest direct effects on LV diastolic distensibility and
370 the onset of LV relaxation in subjects without heart failure. The effects of systemic infusion
371 on diastolic function in this patient group are, however, much greater and involve altered
372 cardiac loading. The overall profile of effect of nitrite may be beneficial in conditions
373 characterized by LV diastolic dysfunction and merits further study.

374

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382

383 **Author Contribution Statement**

384 Conception of the work: AMS, KOG, PC

385 Acquisition and analysis of data: AMS, KOG, AR, AC, MR, LD, HG, NM, PJC, AJW

386 Drafting of the manuscript: AMS, KOG, AJW

387

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393 team for their assistance and support.

394

395

396 **Conflict of Interest**

397 The authors have no conflicts of interest to declare.

398

399

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402 disease. *Nat Rev Cardiol.* 2018;**15**:292-316.
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542

543 **Figure legends**

544

545 **Figure 1.** Effect of intracoronary nitrite on parameters of LV function. **A:** Heart rate; **B:**
546 MAP, mean arterial pressure; **C:** LVEDV, LV end-diastolic volume; **D:** LVESV, LV end-
547 systolic volume; **E:** Ees, end-systolic elastance; **F:** SW, stroke work; **G:** dP/dt_{max} ; **H:**
548 dP/dt_{min} ; **I:** LVEDP, LV end-diastolic pressure; **J:** EDPVR, end-diastolic pressure-volume
549 relation; **K:** LVEST, time to LV end-systole (LVEST); **L:** tau. ****P**<0.01 by 1 way ANOVA
550 with repeated measures and Tukey's post-test. n=17 for LVEST, n=19 for MAP, n=20 for all
551 other parameters.

552

553 **Figure 2.** Effect of intravenous nitrite on parameters of LV function. **A:** Heart rate; **B:** MAP,
554 mean arterial pressure; **C:** LVEDV, end-diastolic volume; **D:** LVESV, end-systolic volume;
555 **E:** Ees, end-systolic elastance; **F:** SW, stroke work; **G:** dP/dt_{max} ; **H:** dP/dt_{min} ; **I:** LVEDP, LV
556 end-diastolic pressure; **J:** EDPVR, end-diastolic pressure-volume relation; **K:** LVEST, time
557 to LV end-systole (LVEST); **L:** tau. ***P**<0.05, ****P**<0.01, *****P**<0.001, ******P**<0.0001 by
558 Student's T test. n=19 for MAP and LVEST, n=20 for all other parameters.

559

560 **Figure 3.** Representative pressure volume loops showing the effect of **(A)** Intracoronary
561 nitrite, **(B)** Intravenous nitrite. Blue loops represent baseline values and orange loops
562 represent response to inorganic nitrite.

563

564 **Figure 4.** Comparison of effect between intracoronary and intravenous nitrite. **A:** EDPVR,
565 End-diastolic pressure volume relationship; **B:** LVEST, Left ventricular electro-systolic time;
566 **C:** dP/dt_{min} ; **D:** tau; **E:** LVEDP, LV end-diastolic pressure; **F:** LVESP, LV end-systolic
567 pressure; **G:** LVEDV, LV end-diastolic volume; **H:** LVESV, time to LV end-systole; **I:**

568 MAP, Mean arterial pressure; **J**: Stroke work; **K**: dP/dt_{max} ; **L**: Starling Contractile Index.

569 ** $P < 0.01$, *** $P < 0.001$ by Student's T test. $n=36$ for LVEST and MAP, $n=40$ for all other

570 parameters.

571

572 **Figure 5.** The effect of nitrite on EF1. **A**, Intracoronary nitrite ($n=20$). **B**, Intravenous nitrite

573 ($n=19$). **C**, Comparison of peak change after intracoronary versus intravenous nitrite. **D**,

574 Representative baseline trace of LV volume (orange) and dP/dt_{max} (blue) with EF1

575 calculation demonstrated. **E**, Representative trace of LV volume (orange) and dP/dt_{max} (blue)

576 following intravenous nitrite with EF1 calculation demonstrated. ** $P < 0.01$ vs baseline,

577 ††† $P < 0.001$ vs intracoronary nitrite by Student's T test.

578

579 **Figure 6.** Correlation between LV mass index (LVMI) and change in LVEDP (**A**, **B**) and

580 between baseline EDPVR and change in LVEDP (**C**, **D**). A and C show data for

581 intracoronary nitrite and B and D show data for intravenous nitrite. $n=20$ for all panels.

582 Statistical analysis by logistical regression.

