Diet-induced obese mouse hearts tolerate an acute high fatty acid exposure that also increases ischemic tolerance

Neoma T. Boardman*, Tina M. Pedersen*, Line Rossvoll, Anne D. Hafstad and Ellen Aasum

Cardiovascular Research Group, Department of Medical Biology, Faculty of Health Sciences, UiT-The Arctic University of Norway, Norway

* N. T. Boardman and T. M. Pedersen contributed equally to this work.

Corresponding Author: Ellen Aasum, ellen.aasum@uit.no

Keywords: heart perfusion, pressure-volume, oxygen consumption, cardiac efficiency, acetylation, mitochondrial respiration

Running head: Acute high fat-load in normal and obese hearts

NEW & NOTEWORTHY. An acute myocardial fat-load leads to oxidative stress, oxygen wasting, mechanical inefficiency, hyperacetylation and impaired mitochondrial function, which can contribute to reduced ischemic tolerance. Following obesity/insulin resistance, hearts were less affected by a high fat-load, which subsequently also improved ischemic tolerance. This study highlights that an acute fat-load affects normal and obese hearts differently, and that obesity renders hearts less vulnerable to the disadvantageous effects of an acute fat-load.

23 ## Abstract

24 An ischemic insult is accompanied by an acute increase in circulating fatty acid (FA) levels, which can
25 induce adverse changes related to cardiac metabolism/energetics. Although chronic hyperlipidemia
26 contributes to the pathogenesis of obesity-/diabetes-related cardiomyopathy, it is unclear how these
27 hearts are affected by an acute high FA-load. We hypothesize that adaptation to chronic FA exposure
28 enhances the obese hearts' ability to handle an acute high FA-load.
29 Diet-induced obese (DIO) and age-matched control (CON) mouse hearts were perfused in the
30 presence of low or high FA-load (0.4 and 1.8 mM). Left ventricular (LV) function, FA oxidation rate,
31 myocardial oxygen consumption and mechanical efficiency were assessed, followed by analysis of
32 myocardial oxidative stress, mitochondrial respiration, protein acetylation as well as gene expression.
33 Finally, ischemic tolerance was determined by examining LV functional recovery and infarct size.
34 Under low FA conditions, DIO hearts showed mild LV dysfunction, oxygen wasting, mechanical
35 inefficiency, and reduced mitochondrial OxPhos. High FA-load increased FA oxidation rates in both
36 groups, but this did not alter any of the above parameters in DIO hearts. In contrast, CON hearts
37 showed FA-induced mechanical inefficiency, oxidative stress and reduced OxPhos, as well as
38 enhanced acetylation and activation of PPARα-dependent gene expression. While high FA-load did
39 not alter functional recovery and infarct size in CON hearts, it increased ischemic tolerance in DIO
40 hearts. Thus, this study demonstrates that acute FA-load affects normal and obese hearts differently,
41 and that chronically elevated circulating FA levels render the DIO heart less vulnerable to the
42 disadvantageous effects of an acute FA-load.

43 ## Introduction

44 An acute myocardial infarction is accompanied by increased circulating FAs, due to adrenergic-driven
45 lipolysis in adipose tissue [36, 42, 43]. An ischemic heart will therefore not only be challenged by
46 hypoxia, but also by an acute high FA-load. In normal hearts, a high FA-load will increase
47 mitochondrial reactive oxygen species (ROS) production and lead to impaired mitochondrial
48 energetics [31, 41], intracellular acidosis [35], as well as Ca^{2+} dysregulation [17, 50] and oxygen
49 wasting with subsequent mechanical inefficiency [9, 27, 28, 40]. These FA-mediated changes could all
50 potentially aggravate ischemia-reperfusion injury, and accordingly high FA levels have been reported
51 to reduce ischemic tolerance in isolated perfused hearts [12, 14, 19, 35].

52 In obesity and diabetes, there is an increased risk of developing heart failure, independent of
53 coronary artery disease and hypertension. This specific cardiomyopathy has been linked to hormonal
54 and metabolic derangements. Accordingly, high circulating levels of insulin, glucose and FAs may
55 contribute in the pathogenesis of diabetic cardiomyopathy. Although preclinical studies generally
56 report reduced ischemic-tolerance in hearts from models of obesity/diabetes, it is less clear how high
57 glucose, insulin and FAs affect these hearts and their tolerance to ischemia. We have previously
58 shown that high glucose and insulin had oxygen sparing effects accompanied by improved post-
59 ischemic functional recovery in hearts from obese diabetic mice [24]. Thus the aim of the present
60 study was to elucidate the effects of an acute high FA-load under similar conditions.

61 It is well known that obese/diabetic hearts undergo metabolic alterations, making them more reliant
62 on FAs as energy substrate [9, 25, 38, 39]. This metabolic shift includes both allosteric control as well
63 as translational and post-translational modifications of metabolic proteins. The enhanced cellular FA
64 processing is due to increased protein and gene expression of FA transporters [20], enzymes related
65 to ß-oxidation [25, 29, 38], as well as higher myocardial FA storage [4]. Although the consensus is
66 that the elevated supply of FAs in obesity/diabetes contributes to the development of cardiac
67 dysfunction, we hypothesized that metabolic adaptation to elevated FA levels in the heart enhance
68 the overall capacity of the heart for handling FAs, and consequently their tolerance to an acute high
69 FA-load.

70 ## Materials and methods

71 **Animals**

72 C57BL/6J male mice (5-6 weeks) were purchased from Charles River Laboratories (Germany). Obesity
73 and insulin resistance were induced by feeding mice an obesogenic diet for 20 weeks (diet-induced
74 obese, DIO) as previously described [25, 39]. Age-matched mice fed a regular chow diet served as
75 controls (CON). All mice were housed in a room with a constant temperature of 23°C and 55%
76 humidity, with a 12:12-h reversed light:dark-cycle, and were given ad libitum access to water and
77 their respective diets. To evaluate insulin resistance, plasma glucose (glucometer, FreeStyle Lite,
78 Alameda, CA), and insulin (commercial kits from DRG Diagnostics, Marburg, Germany) were
79 determined in blood samples obtained from the saphenous vein from fasted (4 hours) mice.
80 Homeostatic model assessment (HOMA) was calculated from fasting blood glucose and insulin levels.
81 Plasma free fatty acids were determined in blood samples taken from the saphenous vein of fed
82 animals prior to euthanasia, using commercial kits from Wako Chemicals (Neuss, Germany). Animal
83 experiments were approved by the Norwegian National Animal Research Authority, which conforms
84 to the National Institute of Health guidelines (NIH publication No. 85-23, revised 1996) and European
85 Directive 2010/63/EU.

86

87 **Assessment of left ventricular (LV) function, fatty acid oxidation and oxygen consumption**

88 Left ventricular (LV) function, fatty acid (FA) oxidation rate and myocardial oxygen consumption
89 (MVO_2) were assessed in isolated perfused hearts. All hearts were perfused in a recirculating mode,
90 using Krebs-Henseleit bicarbonate buffer that contained glucose (5 mM) and either low (0.4 mM) or
91 high (1.8 mM) FA concentration (palmitate prebound to 3% BSA) throughout the entire experiment.
92 High FA levels and FA-load are used interchangeably throughout the manuscript.

93 LV pressure and volume was assessed in working hearts, perfused with either low or high FA
94 concentration, using a conductance catheter (1 F) inserted through the apex [27]. FA oxidation rates
95 were also measured in working hearts using [9,10-^{3}H] palmitate prebound to BSA [1]. MVO_2 was
96 assessed using fiber-optic oxygen probes (FOXY-AL300; Ocean Optics, Duiven, Netherlands) inserted
97 into the perfusion line just above the aortic cannula and into the pulmonary artery to obtain the
98 arterial-venous difference in PO_2.

99 Cardiac power was calculated as the product of left ventricular developed pressure and cardiac
100 output. Mechanical efficiency was calculated by dividing cardiac power by MVO_2. This was expressed
101 as % as both parameters was converted to the same unit. Work-independent MVO_2 was assessed by
102 switching to Langendorff perfusion mode and inserting a vent through the apex. Under these

103 conditions the hearts were paced (7 Hz), and MVO_2 was measured before and after KCl-induced
104 cardiac arrest, representing O_2 cost in unloaded hearts ($MVO_{2\ unloaded}$) and for basal metabolism
105 ($MVO_{2\ BM}$), as previously described [7]. Oxygen cost for processes associated with excitation-
106 contraction coupling (MVO_{2ECC}) were calculated as the difference between $MVO_{2\ unloaded}$ and $MVO_{2\ BM}$.
107

108 **RNA isolation and quantification RT-PCR**

109 LV samples were immersed in RNAlater (Qiagen, Hilden, Germany), and total RNA was extracted
110 using RNeasy Fibrous Tissue protocol (Qiagen). cDNA was obtained from 500 ng RNA, and real-time
111 PCR was performed in a LightCycler®96 System using a 1:6 dilution of the cDNA and FastStart
112 Essential DNA Green Master (Roche). mRNA expression of genes of interest were normalized to the
113 GeNorm value from the three housekeeping genes cyclophilin (*Cyclo*), hypoxanthine guanine
114 phosphoribosyl transferase (*Hprt*), and hydroxymethylbilane synthase (*Hmbs*). Primer sequences for
115 the gene expression of the housekeeping genes, pyruvate dehydrogenase kinase 4 (*Pdk4*), fatty acid
116 tranlocase (*FAT/Cd36*), uncoupling protein 2 and 3 (*Ucp2* and *3*), carnitine palmitoyl transferase 1
117 (*mCpt1*), acyl-CoA thioesterases 2 (*Acot2*), hexokinase 2 (*Hk2*), glutathione peroxidase 3 (*Gpx3*),
118 catalase (*Cat*), superoxide dismutase (*MnSod*) lactate dehydrogenase (*Ldh*), are as previously
119 reported [22, 23]. Additional forward and reverse primer (5'-3') sequences: acyl-CoA thioesterases 1
120 (*Acot1*): forward: AAC-ATC-ACC-TTT-GGA-GGG-GAG, reverse: TCC-CCA-ACC-TCC-AAA-CCA-TCA, acyl-
121 CoA oxidase (*Aox*): forward: GCG-CCA-GTC-TGA-AAT-CAA-G, reverse: ACT-GCT-GCG-TCT-GAA-AAT-
122 CC, glucose transporter 4 (*Glut4*): forward: GAC-GGA-CAC-TCC-ATC-TGT-TG, reverse: GCC-ACG-ATG-
123 GAG-ACA-TAG-C, sirtuin 3 (*Sirt3*): forward: GGC-TCT-ATA-CAC-AGA-ACA-TCG-AC, reverse: GAA-GGA-
124 CCT-TCG-ACA-GAC-CGT, amino acid synthesis 5 like-1 (*GCN5L1/BLOS1*): forward: TCC-CGC-CTG-CTC-
125 AAA-GAA-C reverse: GAG-GTG-ATC-CAC-CAA-CGC-TT.
126

127 **Mitochondrial respiration**

128 Mitochondrial respiration was assessed in isolated cardiac mitochondria from Langendorff perfused
129 hearts subjected to a low or high FA-load for 30 minutes. Respiration was determined by high
130 resolution respirometry using an oxygraph (O2k, Oroboros Instruments, Austria). Pyruvate (5 mM)
131 and malate (2 mM) or palmitoyl-CoA (25µM), L-carnitine (5 mM) and malate (2 mM) served as
132 substrates. Uncoupled respiration (V_0) was assessed in the presence of substrates but without ADP.
133 Coupled respiration (V_{max}, OxPhos) was defined as the peak respiration after adding 100 µmol/L ADP.
134 Respiration rates were adjusted to total protein, and respiratory control ratio (RCR) was calculated as
135 the ratio between V_{max} and V_0. A rate-independent coupling parameter, based on the ratio of
136 molecules of ADP phosphorylated to each oxygen molecule consumed (ADP/O), was also calculated.
137

138 **Mitochondrial protein acetylation**

139 Mitochondrial proteins were lysed in buffer containing: 75 mM Tris HCL, 3.8% SDS, 4 M Urea and
140 cOmplete™ Protease Inhibitor Cocktail (Sigma Aldrich). Laemmli buffer was added and samples were
141 boiled (95°C, 5 minutes). Protein (20 µg) was loaded onto a 15% Criterion gel (Bio Rad) and
142 transferred onto a nitrocellulose membrane (GE Healthcare) following electrophoresis. Membranes
143 were blocked (5% milk, 1 hour), followed by incubation with acetylated Lysine antibody or VDAC
144 antibody (Cell Signalling Technology) overnight at 4°C, and thereafter washed and incubated with
145 anti-Rabbit antibody for 1 hour. Immunopositive bands were developed in Chemoluminescent
146 Peroxidase Substrate 3 (Sigma) for Acetylated Lysine and LumiGlo (Cell Signalling Technology) for
147 VDAC, and visualized using a GE ImageQuant LAS 4000 (GE healthcare). Densitometry of bands was
148 evaluated using Image Studio Protein Lite (LI COR Biosciences) and load was normalized using VDAC
149 as a loading control.

150

151 **Myocardial ROS**

152 Following 30 minutes of Langendorff perfusion, LV tissue was embedded in O.C.T. compound, frozen
153 in cooled isopentane and stored at -70°C. The samples were cryo-sectioned, and stained with
154 dihydroethidium (DHE) for evaluation of superoxide generation using epifluorescence microscope.
155 10-15 images from each heart were obtained for quantification using Image J software.

156

157 **Susceptibility to ischemic injury**

158 Ischemic tolerance was examined in Langendorff perfused hearts. LV function was recorded using an
159 intraventricular fluid-filled balloon connected to a pressure transducer [39]. The volume of the
160 balloon was adjusted to give an end-diastolic pressure of 5-10 mmHg. After 20 minutes of
161 stabilization, the hearts were subjected to 25 min global, no-flow ischemia followed by 90 min
162 reperfusion. Cardiac temperature was continuously monitored and kept at 37±0.5°C throughout the
163 perfusion protocol. At the end of reperfusion, hearts were frozen at -20°C, prior to slicing and
164 staining using a 1% 2,3,5- triphenyl-2H-tetrazolium chloride solution. Infarct size was determined
165 using ImageJ software (National Institutes of Health, Bethesda, MD).

166

167 **Statistical analysis**

168 Data are presented as means ± SE. Differences between two groups were analyzed using an unpaired
169 Student's *t* test. Multiple comparisons were performed by a one-way or two-way ANOVA as indicated
170 in the table or figure legends. When the ANOVA revealed differences, the data sets were compared
171 using Holm-Sidak method as the post hoc test. Differences of P <0.05 were considered significant.

172

173 # Results

174 Diet-induced obese (DIO) mice exhibited higher body weight and increased perirenal fat deposits
175 (Table 1), elevated plasma free fatty acid (FA) levels, and a moderate increase in fasted blood
176 glucose. These mice also showed marked insulin resistance, as indicated by increased HOMA-IR,
177 which was primarily due to higher insulin levels (Table 1).

178

179 **Left ventricular function**

180 Left ventricular (LV) function was assessed in isolated perfused working hearts (Table 2). When
181 perfused with a low FA concentration, hearts from DIO mice are characterized by diastolic
182 dysfunction, as indicated by elevated LV end-diastolic pressure, reduced LV relaxation (Tau), and
183 augmented end-diastolic pressure volume relationship (EDPVR) compared to control hearts perfused
184 under the same conditions. These hearts developed a mild systolic dysfunction, with a small
185 reduction in cardiac output and cardiac power, but without change in prerecruitable stroke work
186 index (PRSWi) (Table 2). Subjecting CON and DIO to a high FA-load (a 4.5-fold increase in FA
187 concentration), did not alter cardiac function in either CON or DIO hearts (Table 2).

188

189 **Myocardial FA oxidation rate and metabolic gene expression**

190 During perfusion with a low FA concentration, myocardial FA oxidation rates were significantly
191 elevated in DIO compared to CON hearts (Figure 1A). This was accompanied by increased expression
192 of FA uptake proteins (FAT/CD36), as well as mitochondrial transport (mCPT1) and oxidation of FAs
193 (ACOX1). We also found the gene expression of PDK4 to be increased, supporting a shift towards
194 enhanced utilization of FAs as energy substrate. In addition, DIO hearts showed increased expression
195 of ACOT1 and 2 as well as UCP2 and 3 (Figure 1B), while gene expression of the proteins GLUT4, HK
196 and LDH was unaltered (Figure 1C).

197 As expected, elevation of the FA concentration in the perfusate increased FA oxidation rates in both
198 CON and DIO hearts. Under these conditions, the rate of FA oxidation was no longer different
199 between the two groups. Interestingly, we found that 30 minutes of high FA perfusion resulted in a
200 marked increase in the expression of PPARα target genes (PDK4, mCPT1, ACOX1, ACOT1 and 2, as
201 well as UCP 2 and 3) in CON hearts, while genes of proteins not under the control of PPARα (HK, LDH
202 and GLUT4) remained unaltered. In DIO hearts, on the other hand, high FA perfusion did not alter the
203 mRNA expression of any of the genes examined, nor did we find PPARα gene expression to be
204 affected by either obesity or by the acute high FA-load (data not shown).

205

206 **Myocardial oxygen consumption and mechanical efficiency**

207 Mechanical efficiency was calculated from cardiac power and MVO_2 in isolated perfused working
208 hearts (Table 2). Under low FA conditions, DIO hearts showed mechanical inefficiency when
209 compared to CON hearts under the same conditions (Figure 2A). Increased MVO_2 in the DIO hearts
210 was also observed when hearts were subjected to unloaded conditions (Figure 2B), which
211 demonstrates a higher O_2 cost for non-mechanical processes in DIO as compared to control hearts,
212 when perfused under low FA conditions. Accordingly, DIO hearts also showed increased O_2 cost for
213 processes related to both basal metabolism ($MVO_{2\ BM}$, Figure 2C) as well as excitation-contraction
214 (EC) coupling ($MVO_{2\ ECC}$) (Figure 2D).

215 The high FA-load markedly decreased mechanical efficiency in CON hearts (Figure 2A). High FAs also
216 increased MVO_2 in unloaded CON hearts (Figure 2B), and we confirmed that high FA-load increased
217 the O_2 cost for both BM (Figure 2C) as well as ECC (Figure 2C). In contrast to CON hearts, the high FA-
218 load neither decreased mechanical efficiency nor increased unloaded MVO_2, nor oxygen cost of BM
219 and ECC in DIO hearts (Figure 2A, B, C and D respectively).

220

221 **Mitochondrial respiration**

222 Mitochondrial respiration was assessed using pyruvate and malate (Figure 3 A-C) or palmitoyl-CoA, L-
223 carnitine and malate (Figure 3 D-E) as substrates for respiration. Mitochondria from DIO hearts
224 perfused with a low FA concentration showed reduced coupled respiration (V_{max}) compared to
225 mitochondria from controls perfused with low FA, both when pyruvate and palmitate served as
226 substrate. Under the same conditions, uncoupled respiration (V_0) was also significantly reduced, such
227 that the respiratory control ratio (RCR) was not different between CON and DIO , regardless of
228 substrate. Correspondingly, the ADP/O ratio was not different between CON and DIO (pyruvate;
229 2.23±0.06 vs. 2.37±0.06; palmitate; 2.08±0.06 vs. 2.09±0.04, in CON and DIO, respectively).

230 When CON hearts were exposed to a high FA-load prior to mitochondrial isolation, V_{max} was
231 significantly reduced, accompanied by a small but not significant reduction in V_0. As the reduction in
232 V_{max} was more pronounced than V_0, RCR was lower in mitochondria from hearts perfused with high
233 as compared to low FAs (palmitate; $p<0.05$, pyruvate; $p=0.067$) (Figure 3). The ADP/O was not altered
234 by subjecting the CON hearts to a high FA-load (2.32±0.03 and 2.16±0.02, pyruvate and palmitate,
235 respectively). In contrast to CON, high FA-load did not attenuate coupled (V_{max}) or uncoupled (V_0)
236 respiration in mitochondria from DIO hearts, regardless of substrate conditions. Thus, both RCR

237 (Figure 3), as well as ADP/O was unaltered by high FAs in DIO (2.32±0.06 and 2.11±0.04, pyruvate and
238 palmitate, respectively).

239

240 **Myocardial ROS content and mitochondrial protein acetylation**

241 ROS levels in LV tissue from DIO was higher than in CON hearts that had been perfused with low FA
242 concentration (Figure 4A). In these hearts, we found increased gene expression of key enzymes in the
243 first line antioxidant defense such as catalase and glutathione peroxidase (*gpx3*), but not mn-
244 superoxide dismutase (*mn-sod*). Subjecting CON hearts to the high FA-load significantly increased
245 ROS levels, and the 30 min high FA perfusion also increased mRNA expression of catalase and GPX3
246 in these hearts (Figure 4B). DIO hearts however, showed a resistance to these FA-induced changes.
247 Interestingly, we did not find ROS content to be different between CON and DIO hearts exposed to a
248 high FA-load.

249 The effect of altered FA concentrations on the lysine acetylation of mitochondrial proteins was also
250 examined. It has previously been shown that diet-induced obesity increases protein acetylation in
251 the obese/diabetic heart [18]. As we did not observe this difference in mitochondria from low FA-
252 perfused hearts, we also isolated mitochondria from hearts perfused without BSA-bound FAs (NoF).
253 Under these conditions, there was a near 2-fold increase in acetylated mitochondrial proteins in DIO
254 hearts compared to CON (Figure 5A).

255 When comparing the acetylation status with different concentrations of FAs, we found a marked
256 dose-dependent increase in protein acetylation in CON hearts (Figure 5B). Interestingly, a similar
257 effect was not observed in DIO hearts (Figure 5C). Gene expression analysis of tissue from these
258 hearts revealed that perfusion with high FA-load did not alter the mRNA expression of sirtuin 3 or
259 GCN5L1 (data not shown).

260

261 **Susceptibility to ischemic injury**

262 Ischemic tolerance was examined in Langendorff perfused hearts, as this perfusion mode is a robust
263 technique for examining myocardial ischemic tolerance. It should be noted, however, that this
264 perfusion mode is less sensitive for picking up LV functional changes [45]. Accordingly, we did not
265 find differences in pre-ischemic function between Langendorff perfused CON and DIO hearts when
266 perfused under low FA conditions (Table 3), even though dysfunction was evident in DIO hearts in
267 the working mode. A significantly lower heart rate was observed in high FA perfused DIO hearts
268 when compared to CON. However, as the lower heart rate was accompanied by higher LV developed

269 pressure (LVDP), the resulting pre-ischemic rate pressure product (RPP) was similar to the other
270 experimental groups (Table 3).

271 All hearts were subjected to 25 minutes of no-flow ischemia followed by reperfusion, with post-
272 ischemic LV function assessed until maximum recovery. Functional recovery (expressed as % of the
273 pre-ischemic values) showed that under low FA conditions, DIO hearts exhibited impaired recovery of
274 RPP (Figure 6C, Table 3) when compared to CON hearts under the same conditions. The impaired
275 recovery was mainly due to a lower recovery of LVDP (Table 3). Corroborating this, infarct size was
276 also greater in DIO hearts under these conditions.

277 Exposing CON hearts to high FA levels did not significantly impair functional recovery, nor was infarct
278 size increased (Figure 6C, Table 3). In contrast, and to our surprise, FA-load was found to significantly
279 improve post-ischemic recovery in DIO hearts, compared to DIO hearts perfused under low FA
280 conditions (Figure 6C). In line with improved post-ischemic functional recovery, infarct size was also
281 reduced following the high FA-load in DIO hearts (Figure 6D). Notably, although DIO hearts showed
282 lower ischemic tolerance when compared to CON hearts when perfused under low FA conditions,
283 there were no significant differences with regards to post-ischemic functional recovery or infarct size
284 between DIO and CON under high FA perfusion (Figure 6).

285 ## Discussion

286 Both physiological and pathophysiological stresses can lead to acute high circulating levels of fatty
287 acids (FAs). Although it has been shown that an acute high FA-load can have adverse effects in the
288 normal heart, to what extent this also occurs in the obese/diabetic heart is less clear. Thus, in the
289 present study we have examined the effects of high FA-load in hearts from diet-induced obese (DIO)
290 mice by comparing this to a low FA condition. DIO hearts perfused under low FA condition displayed
291 the expected metabolic phenotype of diabetic cardiomyopathy (including a high reliance on FA
292 oxidation, increased ROS, and mitochondrial dysfuncion). When exposed to a high FA concentration
293 FA oxidation rate was increased in hearts from both control and DIO mice. This was associated with
294 increased ROS, impaired mitochondrial OxPhos, augmented protein acetylation and altered gene
295 expression only in hearts from controls. In addition, FA-mediated oxygen wasting and mechanical
296 inefficiency only occurred in hearts from normal mice suggesting that high FA-load results in
297 disadvantageous changes in controls but did not further aggravate these parameters in the obese
298 heart. Finally, high FA levels did not affect ischemic tolerance in normal hearts, and we observed
299 increased ischemic tolerance when DIO hearts were exposed to high FAs. These findings clearly show
300 that a high FA-load affects normal and obese hearts differently, and may suggest that chronic
301 hyperlipidemia renders the DIO hearts less vulnerable to the disadvantageous effects of an acute FA-
302 load.

303

304 **High FA-load in the normal heart**

305 In normal hearts, elevation of FAs can have a range of cardiac effects. In the present study, acute
306 high FA-load increased myocardial FA oxidation rate, and likely also intracellular levels of FA and FA
307 intermediates. Accordingly, we found mitochondrial protein acetylation to be increased, suggesting
308 that the acute high FA-load led to a mismatch between the FA supply and oxidation and subsequent
309 acetyl-CoA accumulation. Interestingly, our data also shows that high FA-load markedly increased the
310 expression of PPARα target genes, which supports a FA-mediated activation of PPARα due to
311 increased intracellular FAs levels.

312 The present study confirms a FA-mediated decrease in mechanical efficiency in control hearts, due to
313 an increase in myocardial oxygen consumption (MVO_2) [8, 9, 27]. MVO_2 is determined by both work-
314 dependent factors (pre- and after-load, heart rate, wall stress), and work-independent factors
315 (including myocardial Ca^{2+} control, mitochondrial membrane potential, protein synthesis and
316 transmembrane ionic balance), and we confirmed that the FA-mediated oxygen wasting was
317 primarily linked to work-independent processes [8, 27]. Although this is commonly attributed to the
318 obligatory increase in myocardial FA oxidation (as the oxidation of FAs requires more oxygen for the

319 same amount of ATP produced, compared to glucose), there is, evidence that the increased O_2 cost
320 for FA oxidation *per se* is negligible, as an inhibition of myocardial FA oxidation does not abolish the
321 increase in MVO_2 in normal hearts perfused with high FAs [8, 28].

322 In the present study, the FA-mediated increase in O_2 wasting was accompanied by impaired
323 mitochondrial energetics, as we found reduced OxPhos in mitochondria from high FA-perfused
324 hearts. This suggests that O_2 wasting is not solely explained by excessive mitochondrial respiration
325 but that high FA-load affects extra-mitochondrial processes which contribute to increase MVO_2 as
326 well. Accordingly, we found increased O_2 cost for excitation-contraction (EC) coupling [8], which
327 could be the result of less efficient myocardial Ca^{2+} handling, due to SR Ca^{2+} leak and/or higher
328 energy cost related to sarcolemmal ionic transport [5, 48]. In addition, high FA has been shown to
329 prolong the decay phase of the Ca^{2+} transient in the heart [17]. Although the exact underlying
330 mechanisms linking FA to altered O_2 cost for EC coupling remain to be determined, FA-mediated
331 changes in redox balance [17, 50] may play a role as Ca^{2+} handling proteins are known to be sensitive
332 to redox-linked modifications [32]. In accordance with this, FA-mediated oxidative stress was found
333 to be accompanied by increased expression of catalase and glutathione peroxidase, key enzymes in
334 the first line antioxidant defense.

335

336 **High FA-load in the obese heart**

337 The diet-induced obese (DIO) mice used in the present study resemble a pre-diabetic state that is
338 typically characterized by obesity, elevated circulating free FA levels and insulin resistance. In
339 accordance with our previous studies, left ventricular (LV) pressure-volume recordings revealed that
340 these hearts primarily develop diastolic dysfunction [25, 39, 45], and display mechanical inefficiency
341 due to an increased MVO_2 [25, 39]. This study also confirms that these hearts show higher FA
342 oxidation rates, as well as increased and elevated expression of metabolic genes that are
343 transcriptionally regulated by PPARα in DIO hearts. These include genes encoding for FA transport
344 (CD36, mCPT1) and the regulation of FA and glucose oxidation (ACOX, PDK4), UCP2 and 3 and acyl-
345 CoA thioesterases (ACOT) 1 and 2. The latter is suggested to have a regulatory role in controlling
346 rates of FA oxidation and in subcellular trafficking of FAs. ACOT1 (the cytosolic isoform) catalyzes the
347 cleave of long chain acyl-CoAs into FA and CoA and thus contributes in regulating the ligand (FA)
348 availability for the transcription factor PPARα.

349 The obese heart also showed impaired mitochondrial respiration (OxPhos rate) [2, 25], increased
350 oxidative stress and expression of antioxidant enzymes [2, 25]. The obesity-induced increase in
351 MVO_2 may be related to altered substrate utilization, altered mitochondrial energetics and increased
352 oxidative stress, in addition to structural remodeling and inefficient Ca^{2+} transport (as reviewed in

353 detail by Hafstad *et al.* [21]). In support of the latter, we found augmented O_2 cost for the work-
354 independent processes in the heart [21, 39]. Although some of the changes induced by chronic FA
355 exposure resemble the effects caused by the acute high FA-load in normal hearts, they are not
356 comparable, as long term exposure to high circulating lipid levels seems to make these hearts
357 uniquely adapted to handle high FAs, as discussed in the following section.

358 Despite high FA supply, we did not observe a FA-mediated increase in mitochondrial protein lysine
359 acetylation, nor did we find changes in the expression of PPARα target genes. Together, this suggests
360 that obese hearts have a higher capacity to handle FAs, which may be related to an attenuated FA-
361 mediated accumulation of acetyl-CoA (and thus acetylation) and free FA (and thus PPARα activation).
362 There is also reason to believe that DIO hearts have enhanced capacity for a temporary lipid storage,
363 which will also contribute to limiting the buildup of intracellular FAs and lipid intermediates [4].

364 Although high FA-load did not markedly alter LV function in DIO hearts when they were compared to
365 DIO hearts perfused under low FA conditions, FAs have been reported to improve cell shortening and
366 Ca^{2+} transients in cardiomyocytes from obese and type 2 diabetic mice when compared to conditions
367 where FAs are completely absent [17, 50]. Likewise, the addition of FAs during perfusion of isolated
368 hearts from experimental models of diet-induced obesity [47] and type 2 diabetes [50] has been
369 reported to increase LV function. This effect was reported to be particularly evident in hearts and
370 cardiomyocytes subjected to metabolic (hyperglycemia) and/or adrenergic stress, where the
371 improved function was linked to a FA-mediated increase in the content of reduced glutathione (GSH)
372 and augmented mitochondrial ROS scavenging capacity [10, 50]. These findings suggest that FAs are
373 crucial to ensure a sufficient supply of reducing equivalents to prevent unfavorable ROS production
374 and impaired energetics [50], and that metabolic inflexibility in obese/diabetic hearts may render
375 them energy starved when FAs are omitted in experimental settings of increased cardiac work [6].

376 In accordance with a study by Cole *et al.* [9] using hearts from DIO rats, high FAs (compared to low
377 FAs) did not alter LV mechanical efficiency in DIO mice. Nor did high FA levels alter unloaded MVO_2 or
378 the O_2 cost for EC coupling and BM, which is in accordance with a previous study using type 2
379 diabetic *db/db* mice [7]. We did not find high FA perfusion to alter mitochondrial respiration or
380 coupling efficiency in DIO hearts, nor did it exacerbate myocardial ROS accumulation, corroborating
381 findings in cardiomyocytes from *ob/ob* mice [17] that demonstrated a resistance to a FA-mediated
382 increase in mitochondrial ROS emission [17]. Taken together, the findings in this study suggest that
383 the obese heart is somewhat protected from the potential harmful effects following exposure to an
384 acute high FA-load. Future studies should address myocardial cellular FA processing in the diabetic
385 heart in order to elucidate potential mechanisms which may enhance the capacity for processing FA.

386

387 **The effect of acute high FA-load on ischemic tolerance in normal and obese hearts**

388 Acute myocardial infarction and cardiac surgery is reported to be accompanied by a marked increase
389 in circulating FA levels [36, 42, 43], which is due to a hyper-adrenergic state, leading to increased
390 lipolysis in white adipose tissue. Thus, the exposure to an acute high FA-load poses an additional
391 challenge in the hypoxic state, where FA-mediated O_2 wasting could be particularly detrimental.
392 Accordingly, high FA perfused rat hearts have been reported to have impaired post-ischemic
393 functional recovery when compared to hearts perfused without FAs [12, 14, 19, 35, 37], and
394 inhibition of FA uptake and utilization during ischemia-reperfusion has been associated with
395 improved post-ischemic recovery [31, 35, 41]. Similarly, Dalgas *et al.* recently showed that increasing
396 the FA concentration from 0.4 to 1.2 mM, reduced post-ischemic function and increased infarct size
397 in normal rat hearts [12]. In contrast, we did not find that high FAs exacerbated ischemic injury in
398 normal hearts in the present study despite the disadvantageous effects that occurred in these hearts
399 following FA-load. Although the reason for these discrepancies is not clear, it may be related to the
400 severity of the ischemic insult. As the aim of this study primarily was to examine the effects of high
401 FA-load in obese hearts, the duration of ischemia may have been too short to unmask the
402 disadvantageous effects of high FA levels in the control hearts.

403 In clinical studies, obesity and diabetes are known to worsen the long-term outcome of ischemic
404 heart disease. Although, this can be due to decreased myocardial ischemic tolerance, it can also be
405 due to impaired myocardial reperfusion (due to vascular dysfunction) and/or reduced coronary
406 reserve [49]. Futhermore, clinical studies have shown paradoxical and favorable effects of obesity on
407 the outcome of acute coronary syndrome [3, 11], and studies using animal models of obesity and
408 diabetes have reported both decreased [16, 34], unchanged [26, 33, 53] and even increased [13, 15,
409 29, 30, 51] ischemic tolerance. The discrepancies in preclinical studies could partly be due to
410 differences in the severity of the metabolic disease in these models [44, 46, 51, 53] however, it does
411 not seem to be the sole explanation [15, 26, 52], and differences in perfusion conditions may
412 therefore also play a role. Accordingly, du Toit *et al.* found that high FA levels decreased ischemic
413 tolerance when this was compared to a condition of no FAs in hearts from an obese rat model [14].
414 In contrast, the present study demonstrated that in hearts from obese mice, high FA levels did not
415 worsen, but rather improved the ischemic outcome when compared to a physiologically more
416 relevant FA condition. It should be noted however, that the improved tolerance to ischemia occurred
417 despite an unaltered phenotype. Although, the exact mechanism underlying the protective effects
418 remains to be elucidated, this study highlights a need for more focus on the role and effect of FAs
419 under ischemic conditions which may also contribute to elucidate paradoxical favorable effects of
420 obesity on the outcome of acute coronary syndrome in patients [3, 11].

421

422 **Limitations**

423 Although FAs and glucose are regarded as the main energy substrate to the heart, it should be
424 acknowledged that additional substrates in the perfusion buffer such as lactate, ketone bodies, and
425 branched chain amino acids could potentially have influenced both the ischemic tolerance and the
426 effects of high FAs. In addition, the high FA concentration used in this study (1.8 mM) during
427 perfusion, is at the upper range of the reported levels in patients with an acute myocardial ischemia
428 [43]. However, it should be noted that the high FA concentration used here did not have any adverse
429 effects on cardiac function. Finally, we recognize that this study has not identified the exact
430 underlying mechanism related to the improved tolerance to an acute high FA-load in obese hearts.
431 However, given the important role of FAs in cardiomyocytes (both in physiological and
432 pathophysiological processes), we believe that this is clearly multifactorial and complex (as implied in
433 this study), and will need to be addressed in experiments to come.

434

435 **Concluding remarks**

436 This study confirms that exposure of normal hearts to high (as compared to low) FA levels, induces
437 oxygen wasting, mechanical inefficiency, increased ROS content, hyperacetylation and reduced
438 OxPhos (as summarized in Figure 7). Apart from transcriptional regulation of the metabolic
439 machinery, other FA-mediated processes, such as increased ROS and protein acetylation, may also
440 play a signaling role in the adaptation to match FA oxidation to the increased supply. These factors
441 may lead to changes that increase MVO_2, however this does not need to have any negative
442 functional consequences, unless the heart is also challenged by pathophysiological stress. Thus, an
443 acute FA-load may contribute to a reduced ischemic sensitivity in the normal heart. In obesity and
444 insulin resistance, sustained high circulating FA levels is likely to induce similar cardiac changes
445 (Figure 7). Although the chronic dyslipidemia and FA-mediated changes eventually may contribute in
446 the pathogenesis of diabetic cardiomyopathy, emerging data also suggest that FAs are crucial for
447 maintaining redox status, providing reducing equivalents, and hence mitochondrial energetics, in
448 these hearts. The results from the present study suggest that these hearts have enhanced capacity to
449 handle an acute high FA-load, so that upon myocardial infarction, the accompanying high FA-load
450 does not represent an additive stress. Accordingly, high FA-load did not aggravate mechanical
451 efficiency, MVO_2, ROS, acetylation or OxPhos in hearts from DIO mice. The fact that high FA-load
452 improved ischemic tolerance despite unchanged phenotype, implies that there must be other factors
453 contributing to a worsened outcome following obesity. The therapeutic potential of targeting FA

454 metabolism remains to be determined, however, this study highlights that future studies should
455 focus on the importance of FAs beyond simply serving as an energy substrate for the heart.
456

457 **Acknowledgments:** We acknowledge Trine Lund and Thomas Andreasen for their technical
458 assistance, Matt Kerr at the University of Oxford for guidance related to the protein acetylation
459 analysis, and Terje S. Larsen for his valuable discussion related to the work.
460

461 **Funding:** This work was supported by grants from Norwegian Health Association (NTB and TMP), The
462 Novo Nordisk Foundation (NNF15OC0016244), Norwegian Diabetes Association, UiT-Arctic University
463 of Norway (LR).
464

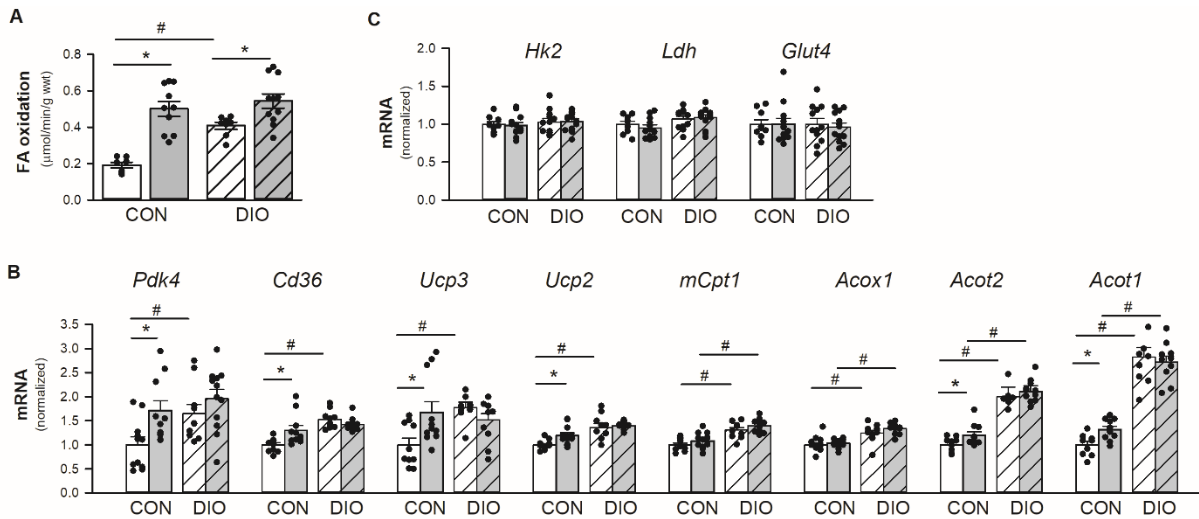
465 **Author contributions:** NTB, TMP, ADH, and EA conceived and designed the research. NTB, TMP, ADH
466 and LR performed experiments and analyzed data. EA, NTB and TMP prepared figures, drafted
467 manuscript, edited and revised manuscript. All authors approved the final version of the manuscript.
468

469 **Conflict of Interest:** The authors declare that the research was conducted in the absence of any
470 commercial or financial relationships that could be construed as a potential conflict of interest.
471

472

473

474 ## Figure legends

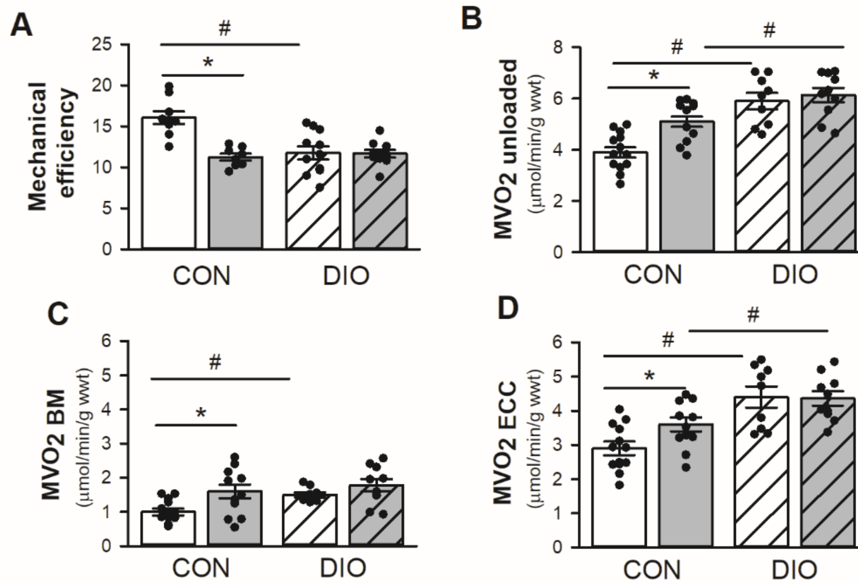


475

476

477 **Figure 1.** FA oxidation rate (A) and mRNA expression (B and C) in hearts from control (CON) and diet-
478 induced obese (DIO) mice, perfused with low (white bars) or high (gray bars) FA concentration. **A:**
479 Myocardial FA oxidation rate (n=7-11) measured in working hearts. **B and C**: Expression of cardiac
480 fuel metabolism genes (n=10-13) in hearts pre-perfused for 30 minutes with low or high FA
481 concentration. This includes gene expression of pyruvate dehydrogenase kinase 4 (*Pdk4*), fatty acid
482 translocase (*FAT/Cd36*), uncoupling protein 2 and 3 (*Ucp2* and *3*), carnitine palmitoyl transferase 1
483 (*mCpt1*), acyl-CoA oxidase 1 (*Acox1*), acyl-CoA thioesterases 1 and 2 (*Acot1* and *2*), hexokinase 2
484 (*Hk2*), lactate dehydrogenase (*Ldh*), and glucose transporter 4 (*Glut4*). Gene expression is normalized
485 to low FA perfused CON hearts. Data are means ± SE. Data were analysed with a two-way ANOVA
486 with Holm-Sidak method as the post hoc test. *p < 0.05 vs low FA, #p < 0.05 vs. CON at the same FA
487 concentration.

488

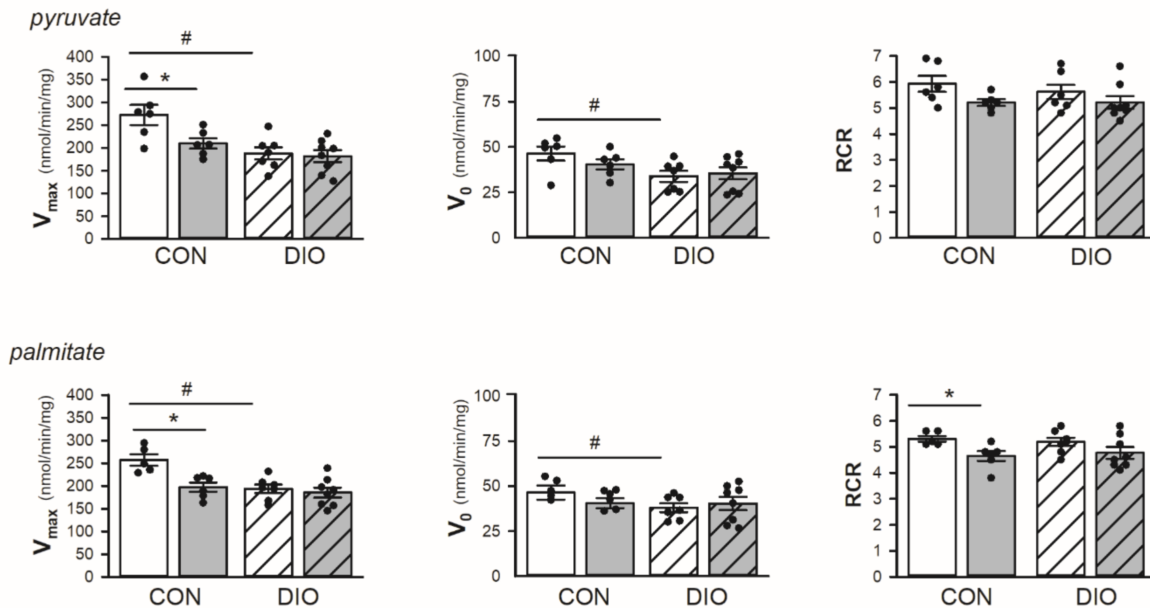


489

490

491 **Figure 2.** Mechanical efficiency (A) was determined by measuring cardiac power and myocardial
492 oxygen consumption (MVO_2, n=8-11) in isolated perfused working hearts from control (CON) and
493 diet-induced obese (DIO) mice perfused with a low (white bars) or high (gray bars) FA concentration.
494 MVO_2 was also measured in electrically paced (7 Hz), unloaded Langendorff perfused hearts (n=9-13)
495 prior to and after KCl-induced cardiac arrest, representing the oxygen cost of the heart in an
496 unloaded condition (B; $MVO_{2\ unloaded}$) and for basal metabolism (C; $MVO_{2\ BM}$), respectively. The oxygen
497 cost for excitation contraction coupling (D; $MVO_{2\ ECC}$) was calculated from the difference between
498 $MVO_{2\ unloaded}$ and $MVO_{2\ BM}$. Data were analysed with a two-way ANOVA with Holm-Sidak method as
499 the post hoc test. Data are means ± SE. *$p < 0.05$ vs. low FA. #$p < 0.05$ vs. CON at the same FA
500 concentration.

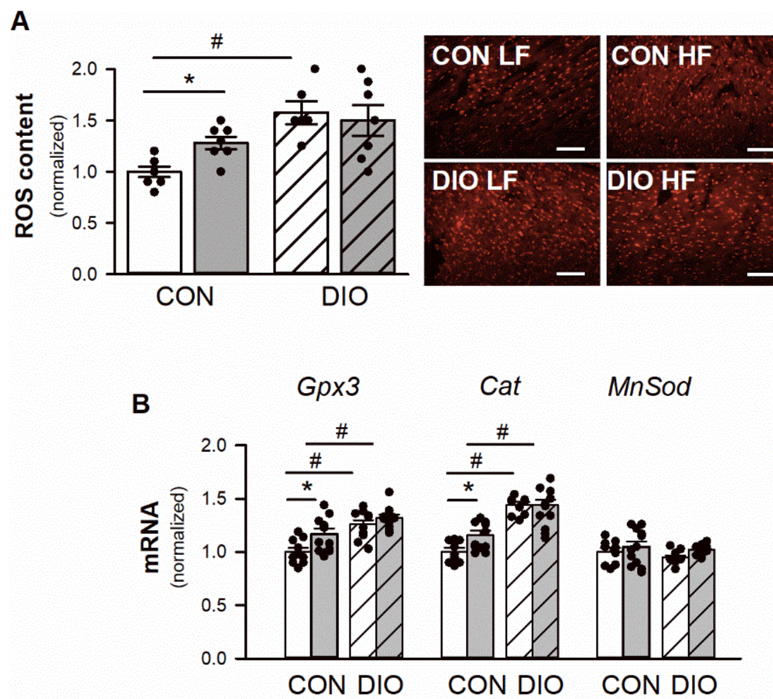
501



502

503 **Figure 3.** Mitochondrial respiration measured in cardiac mitochondria isolated from control (CON,
504 n=5-6) and diet-induced obese (DIO, n=7-8) hearts perfused with low (white bars) or high (gray bars)
505 FA concentration prior to the isolation procedure. The respiratory medium contained 5 mM pyruvate
506 and 2 mM malate **(A-C)** or 25 µM palmitoyl CoA, 5 mM L-carnitine and 2 mM malate **(D-F)**. V_0
507 respiration is defined as the respiratory state before ADP is added and V_{max} is defined as the
508 respiration peak after adding 100 µmol/L ADP. Respiratory control ratio (RCR) was calculated as the
509 ratio between V_{max} and V_0. Mitochondrial respiration rates were normalized to protein. Data were
510 analysed with a two-way ANOVA with Holm Sidak method as the post hoc test. Data are means ±
511 SE. *p < 0.05 vs. low FA. #p < 0.05 vs. CON at the same FA concentration.

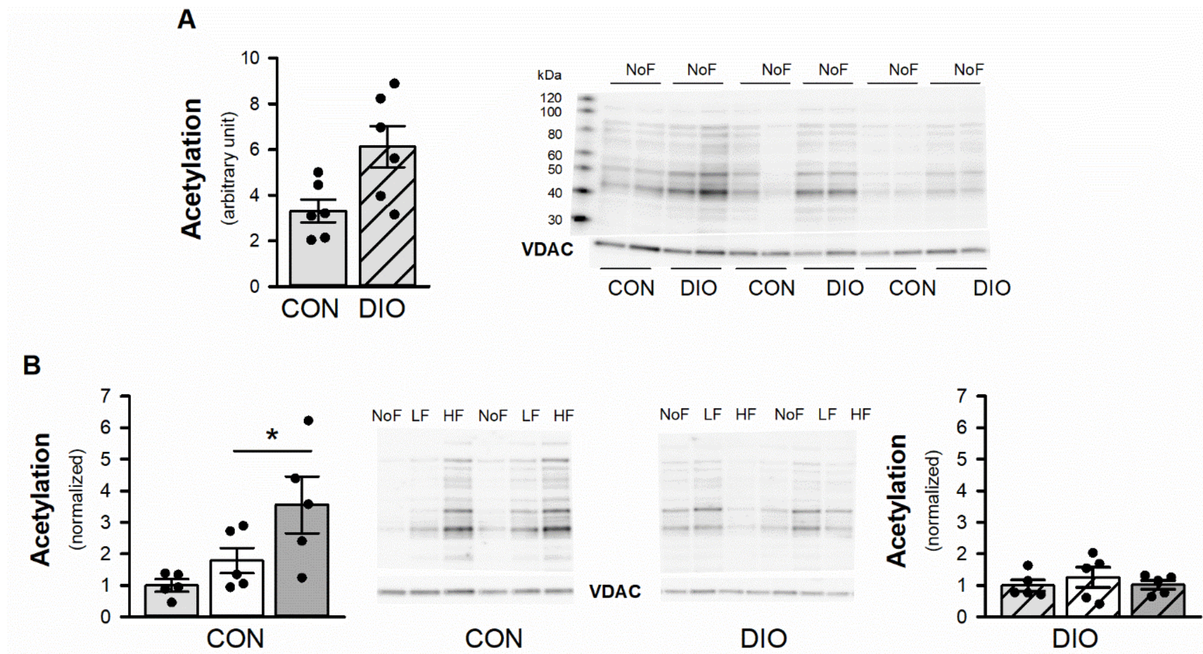
512



513

514 **Figure 4.** Myocardial ROS content and mRNA expression control (CON) and diet-induced obese (DIO)
515 mouse hearts. **A:** Myocardial ROS content (n=6-7) was quantified as fluorescence intensity in left
516 ventricular cryosections using dihydroethidium staining (scale bar: 100 µm). Data were analysed with
517 a two-way ANOVA with Holm-Sidak method as the post hoc test. **B**: Expression of enymes in
518 antioxidant systems (n=10-13) in LV tissue pre-perfused for 30 minutes with low or high FA
519 concentration. This includes gene expression of catalase (*Cat*), glutathione peroxidase (*GPx3*) and
520 Mn-superoxide dismutase (*MnSod*). Data are means ± SE. *p < 0.05 vs. low FA. #p < 0.05 vs. CON at
521 the same FA concentration.

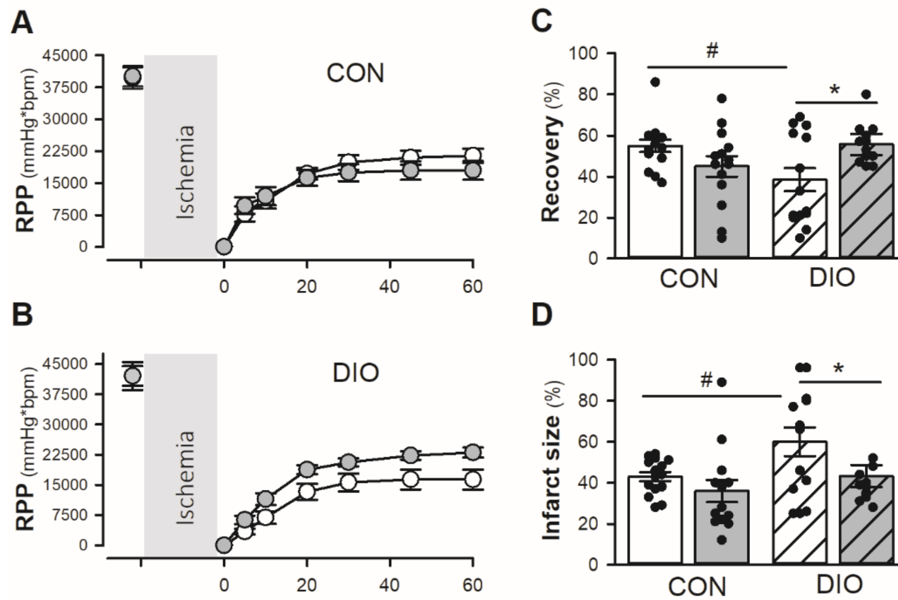
522



523

524 **Figure 5.** Overall mitochondrial protein lysine acetylation in control (CON) and diet-induced obese
525 (DIO) mouse hearts. **A:** Protein acetylation in mitochondria isolated from CON hearts and DIO hearts
526 following perfusion without fatty acids (NoF, light gray bars, n=6). **B:** Protein acetylation in
527 mitochondria isolated from CON hearts and DIO hearts following perfusion without FAs (NoF, light
528 gray bars, n=6), low FAs (LF, white bars, n=6) or high FAs (HF, dark gray bars, n=6). Data in B and C
529 were normalized to NoF within each group (same data as in A), and analysed with a one-way ANOVA
530 with Holm-Sidak method as the post hoc test. Data are means ± SE. *p < 0.05 vs. low FA.

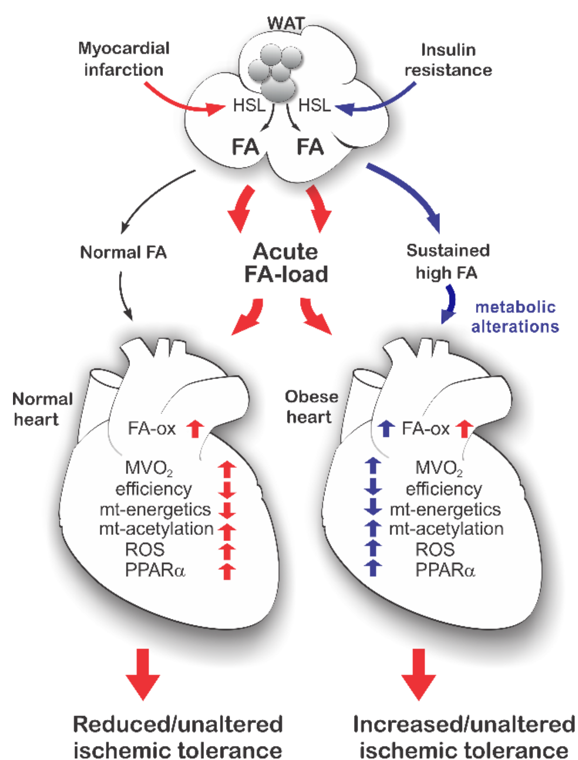
531



532

533 **Figure 6.** Rate pressure product (RPP) (**A and B**), the calculated post-ischemic recovery (**C,** in % of
534 pre-ischemic RPP values, and infarct size (**D**) in isolated perfused hearts from control (CON, n=13-15)
535 and diet-induced obese (DIO, n=12-14) mice. Hearts were subjected to perfusion with a low (white
536 circles and bars) or high (gray circles and bars) FA concentration. Data were analysed with a two-way
537 ANOVA with Holm-Sidak method as the post hoc test. Data are means ± SE. *p < 0.05 vs. low FA. #p
538 < 0.05 vs. CON at the same FA concentration.

539

540 

541

542 **Figure 7.** While a normal heart is exposed to normal circulating fatty acid (FA) levels, obesity-related
543 insulin resistance and lack of insulin-induced inhibition of the hormone sensitive lipase (HSL) in white
544 adipose tissue (WAT), leaves the obese heart exposed to sustained high FA levels (blue arrows). This
545 dyslipidemia alters the metabolic phenotype in obese hearts, and contributes to the development of
546 obesity-/diabetes-related cardiomyopathy. Under acute pathophysiological stress, such as a
547 myocardial infarction, an adrenergic activation of HSL will lead to an acute high FA-load (red arrows).
548 This high FA-load is considered unfavorable in normal hearts, due to FA-mediated changes in
549 myocardial oxygen consumption (MVO_2), mechanical efficiency, ROS production and mitochondrial
550 (mt) energetics. Following obesity and insulin resistance, however, the metabolic alterations induced
551 by the sustained high FA exposure, will enhance these hearts' ability to handle an acute high FA-load,
552 so that is does not futher alter the phenotype of these hearts. Thus, while an acute high FA-load can
553 reduce the ischemic tolerance in normal hearts, it does not represent an additive stress in obese
554 hearts.

555 # References

556

557 1. Aasum E, Hafstad AD, Severson DL, Larsen TS (2003) Age-dependent changes in metabolism,
558 contractile function, and ischemic sensitivity in hearts from db/db mice. Diabetes 52:434-441.
559 doi:10.2337/diabetes.52.2.434

560 2. Anderson EJ, Kypson AP, Rodriguez E, Anderson CA, Lehr EJ, Neufer PD (2009) Substrate-
561 specific derangements in mitochondrial metabolism and redox balance in the atrium of the
562 type 2 diabetic human heart. J Am Coll Cardiol 54:1891-1898. doi:10.1016/j.jacc.2009.07.031

563 3. Angeras O, Albertsson P, Karason K, Ramunddal T, Matejka G, James S, Lagerqvist B, Rosengren
564 A, Omerovic E (2013) Evidence for obesity paradox in patients with acute coronary syndromes:
565 a report from the Swedish Coronary Angiography and Angioplasty Registry. Eur Heart J 34:345-
566 353. doi:10.1093/eurheartj/ehs217

567 4. Aon MA, Bhatt N, Cortassa SC (2014) Mitochondrial and cellular mechanisms for managing
568 lipid excess. Front Physiol 5:282. doi:10.3389/fphys.2014.00282

569 5. Belke DD, Swanson EA, Dillmann WH (2004) Decreased sarcoplasmic reticulum activity and
570 contractility in diabetic db/db mouse heart. Diabetes 53:3201-3208.
571 doi:10.2337/diabetes.53.12.3201

572 6. Bertero E, Sequeira V, Maack C (2018) Let's face the fats: palmitate restores cellular redox
573 state in the diabetic heart. J Physiol. doi:10.1113/JP277473

574 7. Boardman N, Hafstad AD, Larsen TS, Severson DL, Aasum E (2009) Increased O2 cost of basal
575 metabolism and excitation-contraction coupling in hearts from type 2 diabetic mice.
576 Am.J.Physiol Heart Circ.Physiol 296:H1373-H1379. doi: 10.1152/ajpheart.01264.2008

577 8. Boardman NT, Larsen TS, Severson DL, Essop MF, Aasum E (2011) Chronic and acute exposure
578 of mouse hearts to fatty acids increases oxygen cost of excitation-contraction coupling.
579 Am.J.Physiol Heart Circ.Physiol 300:H1631-H1636. doi:10.1152/ajpheart.01190.2010

580 9. Cole MA, Murray AJ, Cochlin LE, Heather LC, McAleese S, Knight NS, Sutton E, Jamil AA,
581 Parassol N, Clarke K (2011) A high fat diet increases mitochondrial fatty acid oxidation and
582 uncoupling to decrease efficiency in rat heart. Basic Res Cardiol 106:447-457.
583 doi:10.1007/s00395-011-0156-1

584 10. Cortassa S, Caceres V, Tocchetti CG, Bernier M, de Cabo R, Paolocci N, Sollott SJ, Aon MA
585 (2018) Metabolic remodelling of glucose, fatty acid and redox pathways in the heart of type 2
586 diabetic mice. J Physiol. doi:10.1113/JP276824

587 11. Curtis JP, Selter JG, Wang Y, Rathore SS, Jovin IS, Jadbabaie F, Kosiborod M, Portnay EL, Sokol
588 SI, Bader F, Krumholz HM (2005) The obesity paradox: body mass index and outcomes in
589 patients with heart failure. Arch Intern Med 165:55-61. doi:10.1001/archinte.165.1.55

590 12. Dalgas C, Povlsen JA, Lofgren B, Erichsen SB, Botker HE (2012) Effects of fatty acids on
591 cardioprotection by pre-ischaemic inhibition of the malate-aspartate shuttle. Clin Exp
592 Pharmacol Physiol 39:878-885. doi:10.1111/j.1440-1681.2012.05749.x

593 13. Donner D, Headrick JP, Peart JN, du Toit EF (2013) Obesity improves myocardial ischaemic
594 tolerance and RISK signalling in insulin-insensitive rats. Dis Model Mech 6:457-466.
595 doi:10.1242/dmm.010959

596 14. du Toit EF, Smith W, Muller C, Strijdom H, Stouthammer B, Woodiwiss AJ, Norton GR, Lochner
597 A (2008) Myocardial susceptibility to ischemic-reperfusion injury in a prediabetic model of
598 dietary-induced obesity. Am.J Physiol Heart Circ.Physiol 294:H2336-H2343.
599 doi:10.1152/ajpheart.00481.2007

600 15. Edland F, Wergeland A, Kopperud R, Asrud KS, Hoivik EA, Witso SL, R AE, Madsen L, Kristiansen
601 K, Bakke M, Doskeland SO, Jonassen AK (2016) Long-term consumption of an obesogenic high
602 fat diet prior to ischemia-reperfusion mediates cardioprotection via Epac1-dependent
603 signaling. Nutr Metab (Lond) 13:87. doi:10.1186/s12986-016-0147-1

604 16. Essop MF, Anna Chan WY, Valle A, Garcia-Palmer FJ, Du Toit EF (2009) Impaired contractile
605 function and mitochondrial respiratory capacity in response to oxygen deprivation in a rat
606 model of pre-diabetes. Acta Physiol (Oxf) 197:289-296. doi:10.1111/j.1748-1716.2009.02024.x

607 17. Fauconnier J, Andersson DC, Zhang SJ, Lanner JT, Wibom R, Katz A, Bruton JD, Westerblad H
608 (2007) Effects of palmitate on Ca(2+) handling in adult control and ob/ob cardiomyocytes:
609 impact of mitochondrial reactive oxygen species. Diabetes 56:1136-1142. doi:10.2337/db06-
610 0739

611 18. Fukushima A, Alrob OA, Zhang L, Wagg CS, Altamimi T, Rawat S, Rebeyka IM, Kantor PF,
612 Lopaschuk GD (2016) Acetylation and succinylation contribute to maturational alterations in
613 energy metabolism in the newborn heart. Am J Physiol Heart Circ Physiol 311:H347-363.
614 doi:10.1152/ajpheart.00900.2015

615 19. Gambert S, Vergely C, Filomenko R, Moreau D, Bettaieb A, Opie LH, Rochette L (2006) Adverse
616 effects of free fatty acid associated with increased oxidative stress in postischemic isolated rat
617 hearts. Mol Cell Biochem 283:147-152. doi:10.1007/s11010-006-2518-9

618 20. Glatz JF, Luiken JJ, Bonen A (2010) Membrane fatty acid transporters as regulators of lipid
619 metabolism: implications for metabolic disease. Physiol Rev 90:367-417.
620 doi:10.1152/physrev.00003.2009

621 21. Hafstad AD, Boardman N, Aasum E (2015) How exercise may amend metabolic disturbances in
622 diabetic cardiomyopathy. Antioxid Redox Signal 22:1587-1605. doi:10.1089/ars.2015.6304

623 22. Hafstad AD, Boardman NT, Lund J, Hagve M, Khalid AM, Wisloff U, Larsen TS, Aasum E (2011)
624 High intensity interval training alters substrate utilization and reduces oxygen consumption in
625 the heart. J.Appl.Physiol (1985.) 111:1235-1241. doi:10.1093/cvr/cvp132

626 23. Hafstad AD, Khalid AM, Hagve M, Lund T, Larsen TS, Severson DL, Clarke K, Berge RK, Aasum E
627 (2009) Cardiac peroxisome proliferator-activated receptor-alpha activation causes increased
628 fatty acid oxidation, reducing efficiency and post-ischaemic functional loss. Cardiovasc Res
629 83:519-526. doi:10.1093/cvr/cvp132

630 24. Hafstad AD, Khalid AM, How OJ, Larsen TS, Aasum E (2007) Glucose and insulin improve
631 cardiac efficiency and postischemic functional recovery in perfused hearts from type 2 diabetic
632 (db/db) mice. Am J Physiol Endocrinol Metab 292:E1288-1294.
633 doi:10.1152/ajpendo.00504.2006

634 25. Hafstad AD, Lund J, Hadler-Olsen E, Hoper AC, Larsen TS, Aasum E (2013) High- and moderate-
635 intensity training normalizes ventricular function and mechanoenergetics in mice with diet-
636 induced obesity. Diabetes 62:2287-2294. doi:10.2337/db12-1580

637 26. Hjortbak MV, Hjort J, Povlsen JA, Jensen RV, Stottrup NB, Laursen MR, Jespersen NR, Lofgren B,
638 Botker HE (2018) Influence of diabetes mellitus duration on the efficacy of ischemic
639 preconditioning in a Zucker diabetic fatty rat model. PLoS One 13:e0192981.
640 doi:10.1371/journal.pone.0192981

641 27. How OJ, Aasum E, Kunnathu S, Severson DL, Myhre ES, Larsen TS (2005) Influence of substrate
642 supply on cardiac efficiency, as measured by pressure-volume analysis in ex vivo mouse hearts.
643 Am.J.Physiol Heart Circ.Physiol 288:H2979-H2985. doi:10.1152/ajpheart.00084.2005

644 28. Hutter JF, Piper HM, Spieckerman PG (1985) Effect of fatty acid oxidation on efficiency of
645 energy production in rat heart. Am.J Physiol 249:H723-H728.
646 doi:10.1152/ajpheart.1985.249.4.H723

647 29. Inserte J, Aluja D, Barba I, Ruiz-Meana M, Miro E, Poncelas M, Vilardosa U, Castellano J, Garcia-
648 Dorado D (2019) High-fat diet improves tolerance to myocardial ischemia by delaying
649 normalization of intracellular PH at reperfusion. J Mol Cell Cardiol 133:164-173.
650 doi:10.1016/j.yjmcc.2019.06.001

651 30. Jansen KM, Moreno S, Garcia-Roves PM, Larsen TS (2019) Dietary Calanus oil recovers
652 metabolic flexibility and rescues postischemic cardiac function in obese female mice. Am J
653 Physiol Heart Circ Physiol 317:H290-H299. doi:10.1152/ajpheart.00191.2019

654 31. Jaswal JS, Keung W, Wang W, Ussher JR, Lopaschuk GD (2011) Targeting fatty acid and
655 carbohydrate oxidation--a novel therapeutic intervention in the ischemic and failing heart.
656 Biochim Biophys Acta 1813:1333-1350. doi:10.1016/j.bbamcr.2011.01.015

657 32. Kuster GM, Lancel S, Zhang J, Communal C, Trucillo MP, Lim CC, Pfister O, Weinberg EO, Cohen
658 RA, Liao R, Siwik DA, Colucci WS (2010) Redox-mediated reciprocal regulation of SERCA and
659 Na+-Ca2+ exchanger contributes to sarcoplasmic reticulum Ca2+ depletion in cardiac
660 myocytes. Free Radic Biol Med 48:1182-1187. doi:10.1016/j.freeradbiomed.2010.01.038

661 33. Lacerda L, Opie LH, Lecour S (2012) Influence of tumour necrosis factor alpha on the outcome
662 of ischaemic postconditioning in the presence of obesity and diabetes. Exp Diabetes Res
663 2012:502654. doi:10.1155/2012/502654

664 34. Littlejohns B, Pasdois P, Duggan S, Bond AR, Heesom K, Jackson CL, Angelini GD, Halestrap AP,
665 Suleiman MS (2014) Hearts from mice fed a non-obesogenic high-fat diet exhibit changes in
666 their oxidative state, calcium and mitochondria in parallel with increased susceptibility to
667 reperfusion injury. PLoS One 9:e100579. doi:10.1371/journal.pone.0100579

668 35. Liu Q, Docherty JC, Rendell JC, Clanachan AS, Lopaschuk GD (2002) High levels of fatty acids
669 delay the recovery of intracellular pH and cardiac efficiency in post-ischemic hearts by
670 inhibiting glucose oxidation. J.Am.Coll.Cardiol. 39:718-725. doi:10.1016/s0735-1097(01)01803-
671 4

672 36. Lopaschuk GD, Collins Nakai R, Olley PM, Montague TJ, McNeil G (1994) Plasma fatty acid
673 levels in infants and adults after myocardial ischemia. Am.Heart J. 128:61-67.
674 doi:10.1016/0002-8703(94)90010-8

675 37. Lopaschuk GD, Saddik M, Barr R, Huang L, Barker CC, Muzyka RA (1992) Effects of high levels of
676 fatty acids on functional recovery of ischemic hearts from diabetic rats. Am J Physiol
677 263:E1046-1053. doi:10.1152/ajpendo.2006.263.6.E1046

678 38. Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, Stanley WC (2010) Myocardial fatty acid
679 metabolism in health and disease. Physiol Rev. 90:207-258. doi:10.1152/physrev.00015.2009

680 39. Lund J, Hafstad AD, Boardman NT, Rossvoll L, Rolim NP, Ahmed MS, Florholmen G, Attramadal
681 H, Wisloff U, Larsen TS, Aasum E (2015) Exercise training promotes cardioprotection through
682 oxygen-sparing action in high fat-fed mice. Am.J.Physiol Heart Circ.Physiol 308:H823-H829.
683 doi:10.1152/ajpheart.00734.2014

684 40. Mjos OD (1971) Effect of free fatty acids on myocardial function and oxygen consumption in
685 intact dogs. J Clin Invest 50:1386-1389. doi:10.1172/JCI106621

686 41. Nagendran J, Pulinilkunnil T, Kienesberger PC, Sung MM, Fung D, Febbraio M, Dyck JR (2013)
687 Cardiomyocyte-specific ablation of CD36 improves post-ischemic functional recovery. J Mol
688 Cell Cardiol 63:180-188. doi:10.1016/j.yjmcc.2013.07.020

689 42. Oliver MF, Kurien VA, Greenwood TW (1968) Relation between serum-free-fatty acids and
690 arrhythmias and death after acute myocardial infarction. Lancet 1:710-714.
691 doi:10.1016/s0140-6736(68)92163-6

692 43. Opie LH (1975) Metabolism of free fatty acids, glucose and catecholamines in acute myocardial
693 infarction. Relation to myocardial ischemia and infarct size. Am J Cardiol 36:938-953.
694 doi:10.1016/0002-9149(75)90086-7

695 44. Paelestik KB, Jespersen NR, Jensen RV, Johnsen J, Botker HE, Kristiansen SB (2017) Effects of
696 hypoglycemia on myocardial susceptibility to ischemia-reperfusion injury and preconditioning
697 in hearts from rats with and without type 2 diabetes. Cardiovasc Diabetol 16:148.
698 doi:10.1186/s12933-017-0628-1

699 45. Pedersen TM, Boardman NT, Hafstad AD, Aasum E (2018) Isolated perfused working hearts
700 provide valuable additional information during phenotypic assessment of the diabetic mouse
701 heart. PLoS One 13:e0204843. doi:10.1371/journal.pone.0204843

702 46. Povlsen JA, Lofgren B, Dalgas C, Birkler RI, Johannsen M, Stottrup NB, Botker HE (2013)
703 Protection against myocardial ischemia-reperfusion injury at onset of type 2 diabetes in Zucker
704 diabetic fatty rats is associated with altered glucose oxidation. PLoS One 8:e64093.
705 doi:10.1371/journal.pone.0064093

706 47. Smith W, Norton GR, Woodiwiss AJ, Lochner A, du Toit EF (2016) Dependence of Cardiac
707 Systolic Function on Elevated Fatty Acid Availability in Obese, Insulin-Resistant Rats. J Card Fail
708 22:560-568. doi:10.1016/j.cardfail.2016.04.012

709 48. Stolen TO, Hoydal MA, Kemi OJ, Catalucci D, Ceci M, Aasum E, Larsen T, Rolim N, Condorelli G,
710 Smith GL, Wisloff U (2009) Interval training normalizes cardiomyocyte function, diastolic Ca2+
711 control, and SR Ca2+ release synchronicity in a mouse model of diabetic cardiomyopathy.
712 Circulation Research 105:527-536. doi:10.1161/CIRCRESAHA.109.199810

713 49. Stone PH, Muller JE, Hartwell T, York BJ, Rutherford JD, Parker CB, Turi ZG, Strauss HW,
714 Willerson JT, Robertson T, et al. (1989) The effect of diabetes mellitus on prognosis and serial
715 left ventricular function after acute myocardial infarction: contribution of both coronary

716 disease and diastolic left ventricular dysfunction to the adverse prognosis. The MILIS Study
717 Group. J Am Coll Cardiol 14:49-57. doi:10.1016/0735-1097(89)90053-3

718 50. Tocchetti CG, Caceres V, Stanley BA, Xie C, Shi S, Watson WH, O'Rourke B, Spadari-Bratfisch RC,
719 Cortassa S, Akar FG, Paolocci N, Aon MA (2012) GSH or palmitate preserves mitochondrial
720 energetic/redox balance, preventing mechanical dysfunction in metabolically challenged
721 myocytes/hearts from type 2 diabetic mice. Diabetes 61:3094-3105. doi:10.2337/db12-0072

722 51. Webster I, Salie R, Marais E, Fan WJ, Maarman G, Huisamen B, Lochner A (2017) Myocardial
723 susceptibility to ischaemia/reperfusion in obesity: a re-evaluation of the effects of age. BMC
724 Physiol 17:3. doi:10.1186/s12899-017-0030-y

725 52. Wensley I, Salaveria K, Bulmer AC, Donner DG, du Toit EF (2013) Myocardial structure, function
726 and ischaemic tolerance in a rodent model of obesity with insulin resistance. Exp Physiol
727 98:1552-1564. doi:10.1113/expphysiol.2013.074948

728 53. Whittington HJ, Harding I, Stephenson CI, Bell R, Hausenloy DJ, Mocanu MM, Yellon DM (2013)
729 Cardioprotection in the aging, diabetic heart: the loss of protective Akt signalling. Cardiovasc
730 Res 99:694-704. doi:10.1093/cvr/cvt140

731