1   **Effect of Iron Isomaltoside on Skeletal Muscle Energetics in Patients with Chronic Heart Failure**

2   **and Iron Deficiency: The FERRIC-HF II Randomized Mechanistic Trial**

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4   Geoffrey Charles-Edwards, PhD,[1] Nelson Amaral, MD,[2,3] Alison Sleigh, PhD,[4] Salma Ayis, PhD,[5]

5   Norman Catibog, RDCS,[2,3] Theresa McDonagh, MD, FRCP,[2,3] Mark Monaghan, MSc, PhD [2,3] George

6   Amin-Youssef, MSc, MRCP,[3] Graham J. Kemp, DM, FRCPath,[6]

7   Ajay M. Shah, FRCP, FMedSci, [2,3] Darlington O. Okonko, MBBS, PhD *[2,3]

8   **Affiliations:**

9   [1]School of Imaging Sciences and Biomedical Engineering, King's College, London, UK; Medical

10   Physics, Div. of Imaging Sciences and Biomedical Engineering, Guy's and St Thomas'-NHS

11   Foundation Trust, London, UK; [2]King's College London British Heart Foundation Centre of

12   Excellence, School of Cardiovascular Medicine and Sciences, James Black Centre, London, UK. [3]Dept.

13   of Cardiology, King's College Hospital NHS Foundation Trust, London, UK. [4]Wolfson Brain Imaging

14   Centre, University of Cambridge School of Clinical Medicine, Cambridge, UK; Wellcome Trust-MRC

15   Institute of Metabolic Science, Cambridge, UK; NIHR/Wellcome Trust Clinical Research Facility,

16   Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK; [5] School of Population

17   Health and Environmental Sciences, Kings College London, London, UK; [6] Dept of Musculoskeletal

18   Biology, University of Liverpool and MRC-Arthritis Research UK Centre for Integrated research into

19   Musculoskeletal Ageing (CIMA), Liverpool, UK.

20   **Correspondence:**

21   *Dr Darlington Obinnaya Okonko

22   James Black Centre, 125 Coldharbour Lane, London SE5 9NU, UK

23   obi.okonko@kcl.ac.uk, Tel: 44 (0)207 848 5017

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25 **ABSTRACT**

26 **Background:** Iron repletion augments exercise capacity in chronic heart failure (CHF) but there is a

27 lack of mechanistic data explaining how iron could increment exercise performance despite minimal

28 changes in hemoglobin (Hb). Besides Hb, iron is an obligate component of mitochondrial enzymes that

29 generate cellular energy in the form of adenosine triphosphate and phosphocreatine (PCr). Dynamic

30 phosphorus magnetic resonance spectroscopy (^{31}P MRS) is a noninvasive tool that quantifies *in vivo*

31 muscle energetics by measuring the kinetics of PCr recovery after exertion. We tested the hypothesis

32 that intravenous (IV) iron repletion in CHF enhances skeletal muscle energetics as reflected by shorter

33 PCr recovery half-times (PCr $t_{1/2}$) on ^{31}P MRS.

34

35 **Methods:** We enrolled 40 patients (50% anemic) with CHF, NYHA class≥ II, LVEF≤ 45%, and iron

36 deficiency (ferritin<100μg/L or 100-300μg/L with transferrin saturation [TSAT]< 20%). Subjects

37 underwent stratified (anemic vs non-anemic) randomization (1:1) to a single, double-blinded, total dose

38 infusion of Iron Isomaltoside (IIM) or saline placebo with endpoints reassessed early at 2 weeks post-

39 treatment to minimise confounding from exercise adaptation. The primary endpoint was PCr $t_{1/2}$ at 2

40 weeks. Secondary endpoints included adenosine diphosphate recovery half-time (ADP $t_{1/2;}$ energetic

41 marker), iron status, symptoms, Hb, exercise capacity and safety.

42

43 **Results:** In the total population, treatment groups were similar at baseline. At 2 weeks, IIM improved

44 PCr $t_{1/2}$ (adjusted difference -6.8s [95% confidence interval 11.5,-2.1], P=0.006), ADP $t_{1/2}$ (-5.3s [-9.7,-

45 0.9], P=0.02), ferritin (304ng/mL [217,391], P<0.0001), TSAT (6.8% [2.7,10.8], P=0.002), NYHA

46 class (-0.23[-0.46,-0.01], P=0.04), resting respiratory rate (-0.7 breaths/min [-1.2,-0.2], P=0.009) and

47 post-exercise Borg dyspnea score (-2.0[-3.7,-0.3], P=0.04), but not Hb (2.4g/L [-3.5,8.4], P=0.41).

48 Adverse events were similar between groups. In subgroup analyses, IIM improved PCr $t_{1/2}$ in anemic (-

49 8.4s [-16.7,-0.2], P=0.04) and non-anemic (-5.2s [-10.6,0.2], P=0.06) cohorts.

50   **Conclusion:** In patients with CHF and ID, a total repletion dose of IIM given at a single sitting is well

51   tolerated and associated with faster skeletal muscle PCr $t_{1/2}$ at 2 weeks, implying better mitochondrial

52   function. Augmented skeletal muscle energetics might therefore be an important mechanism via which

53   iron repletion confers benefits in CHF despite minimal Hb changes.

54

55   **Clinical Trial Registration:** URL: https://www.clinicaltrialsregister.eu/ctr-search/trial/2012-005592-

56   13/GB. Unique identifier: EudraCT 2012-005592-13.

57

58 **CLINICAL PERSPECTIVE**

59 **What is New?**

60 • Little is known about how intravenous iron repletion augments exercise capacity in chronic

61 heart failure (CHF) despite minimal hemoglobin changes.

62 • This randomized, double-blind, placebo-controlled Ferric Iron in Heart Failure (FERRIC-HF) II

63 trial shows that a single total dose infusion of Iron Isomaltoside repleted iron stores and

64 augmented skeletal muscle energetics at 2 weeks post-infusion.

65 • Enhancements in skeletal muscle energetics, which imply better mitochondrial function, were

66 accompanied by improved symptoms despite no change in hemogobin at 2 weeks.

67

68 **What are the Clinical Implications?**

69 • Augmented skeletal muscle energetics is a likely mechanism via which iron repletion confers

70 benefits in CHF despite minimal hemoglobin changes.

71 • FERRIC-HF II supports clinical iron repletion in CHF.

72 • A total repletion dose of Iron Isomaltoside given at a single sitting is feasible in CHF patients.

73

74 **INTRODUCTION**

75 Iron deficiency (ID) is prevalent and ominous in chronic heart failure (CHF),[1-4] and its correction

76 markedly improves symptoms and exercise tolerance. Yet, in landmark studies such as the seminal

77 FERRIC-HF (Ferric Iron in Heart Failure) trial, the hemoglobin (Hb) response to intravenous (IV) iron

78 was surprisingly small (5 g/L increase over 4 months),[3,4] and did not correlate with changes in exercise

79 indices. Consequently, it is unknown how IV iron augments exercise capacity despite minimal changes

80 in Hb, and this paucity of mechanistic data partly hampers the clinical uptake of iron repletion in CHF.

81

82 Exercise is a highly energetic process with skeletal muscle contractions powered by the hydrolysis of

83 adenosine triphosphate (ATP) which is buffered by phosphocreatine (PCr) consumption.[5,6] To sustain

84 exertion, PCr is resynthesised via glycolysis and mitochondrial oxidative phosphorylation (OXPHOS)

85 which generates over 95% of cellular ATP.[7,8] Iron is a component of Hb and myoglobin, and of

86 proteins of the Krebs cycle, ß-oxidation pathway, and the electron transport chain which determine

87 OXPHOS capacity.[9-12] In animal studies, cellular ID triggers mitochondrial dysfunction and exercise

88 intolerance, even in the absence of anemia.[13-15] Thus, iron repletion might enhance exercise capacity by

89 energizing skeletal muscle independently of Hb. To date, no clinical trial has addressed this in humans.

90

91 Dynamic phosphorus magnetic resonance spectroscopy (^{31}P MRS) is uniquely suited to quantifying

92 human skeletal muscle energetics *in vivo*.[16,17] It enables the real-time noninvasive tracking of skeletal

93 muscle PCr, ATP and inorganic phosphate (Pi) concentrations and cytostolic pH during exercise and

94 recovery. Because PCr replenishment on cessation of exercise is predominantly driven by OXPHOS,[18]

95 the PCr recovery half-time (PCr $t_{1/2}$) is an inverse marker of mitochondrial oxidative function that is

96 relatively independent of exercise intensity.[16] We conducted the FERRIC-HF II trial to test the

97 hypothesis that iron repletion with a single total dose infusion of Iron Isomaltoside (IIM) would

98 improve skeletal muscle energetics as reflected by a shorter PCr $t_{1/2}$ in CHF patients.

99 **METHODS**

100 **Study Design**

101 FERRIC-HF II was an investigator-led, randomized, double-blind, placebo-controlled, mechanistic trial

102 conducted at King's College Hospital, London, UK. The study involved 1 screening visit, 2 baseline

103 visits within 4 weeks of screening, and 2 final visits 2 weeks after treatment allocation. The protocol

104 was approved by the South-Central Berkshire ethics committee, the UK Medicines and Healthcare

105 products Regulatory Agency, and the independent local research governance board. King's College

106 London University and King's College Hospital NHS Foundation Trust were co-sponsors. The King's

107 Health Partners clinical trial office monitored the trial and ensured compliance with the International

108 Conference on Harmonization guidelines for Good Clinical Practice and the Declaration of Helsinki.

109 Written informed consent was obtained from all patients. The trial is registered on

110 clinicaltrialsregister.eu (EudraCT 2012-005592-13) and data from this analysis is obtainable from the

111 authors on reasonable request.

112

113 **Study Patients**

114 Eligibility criteria were: age ≥30 years; stable symptomatic CHF (New York Heart Association

115 [NYHA] III and left ventricular ejection fraction [LVEF] ≤45%, or if NYHA II then LVEF ≤40%

116 within the preceding 6 months); use of optimal CHF drugs for ≥4 weeks without dose changes for ≥2

117 weeks; screening Hb <120 g/L in females and < 130 g/L in males (anemic group) or ≥120 g/L in

118 females and ≥ 130 g/L in males (non-anemic group); ID as defined by the FERRIC-HF criteria of

119 ferritin <100 μg/L or 100-300 μg/L with transferrin saturation (TSAT) <20%;[3] folate and vitamin B_{12}

120 levels ≥ lower limit of reference range; resting blood pressure ≤160/100 mm Hg; and a negative

121 pregnancy test in women of child-bearing age.

122

123 Exclusion criteria were a history of acquired iron overload, known hemochromatosis or first degree

124 relatives with hemochromatosis; an allergic disorder (e.g., asthma, eczema, and anaphylactic reactions);

125 prior hypersensitivity to IV iron drugs or their excipients; active infection, bleeding, malignancy,

126 hemolytic anemia, rheumatoid arthritis, and myelodysplasia; HIV/AIDS; chronic liver disease with

127 transaminases >3 times the upper limit of the reference range; chronic lung disease with $FEV_1 < 50\%$

128 predicted; coagulopathy or anticoagulated for a metallic valve or LV thrombus; contraindications to

129 MRS; immunosuppressant use; renal dialysis; need for erythropoietin or blood transfusions; unstable

130 angina; severe obstructive cardiac lesions; uncontrolled arrhythmias; and musculoskeletal limitations.

131

132 **Randomization**

133 Qualifying patients attended for 2 baseline visits at week 0 collectively including a clinical history,

134 physical examination, NYHA class assessment, 12-lead electrocardiogram, blood tests, Kansas City

135 Cardiomyopathy Questionnaire (KCCQ), echocardiogram, 6-min walk test, cardiopulmonary exercise

136 test, quadriceps muscle ^{31}P MRS, and a vastus lateralis muscle biopsy. At the end of the second visit,

137 patients were randomly assigned within 2 strata (anemic or non-anemic) in permuted blocks of 4 to a

138 single total repletion dose of IIM or placebo (normal saline) in a 1:1 ratio. Randomization was

139 performed by a clinical trials pharmacist using an automated web-based system (Sealed Envelope Ltd,

140 London, UK).

141

142 **Study Drug and Blinding**

143 Iron (III) isomaltoside 1000 (Monofer®; Pharmacosmos A/S, Holbaek, Denmark) was provided as a

144 solution for IV infusion in 100mg iron/mL ampoules. The total repletion dose was calculated to the

145 nearest multiple of 100mg using the Ganzoni formula: body weight (kg) x 2.4 x (15 - patients

146 Hb[g/dL]) + 500 mg (for stores).[19] Doses of 0-10 mg/kg and 11-20 mg/kg were infused over 30 and 60

147 minutes respectively. Doses exceeding 20 mg/kg were split and given at 2 separate sittings 1 week

148   apart. Monofer® was added to 100 mL sterile 0.9% saline for infusions. Patients randomized to placebo

149   had their repletion dose and infusion duration calculated as above but received 100 mL sterile 0.9%

150   saline over the infusion period. All patients were observed for 1 hour post treatment with heart rate and

151   blood pressure documented every 15 minutes. Patients who received placebo were offered Monofer® at

152   the end of the study if they remained iron-deficient.

153

154   To achieve blinding, allocated therapy was dispensed by a clinical trials pharmacist to unblinded

155   research nurses who prepared and administered the infusions using opaque IV bags and giving sets

156   (Medipak, Virginia, USA). All other members of the research team vacated the infusion room before

157   the allocated therapy was collected from pharmacy. A curtain shielded the infusion arm from the

158   patient. The unblinded nurse was not involved in assessing endpoints.

159

160   **Primary Endpoint**

161   The primary endpoint was skeletal muscle energetics at 2 weeks post-treatment as assessed by the PCr

162     $t_{1/2}$ on dynamic ^{31}P MRS. This was performed on a clinical 3T scanner (Achieva, Philips Medical

163   Systems, Best, the Netherlands). Patients were asked to refrain from strenuous exercise and alcohol for

164   the preceding 24 hours, and from caffeine and food for the preceding 6 and 2 hours, respectively.

165   Heights and weights were measured to the nearest integer and used to calculate lean body mass.[20]

166   Subjects were positioned feet-first and supine in the scanner and a 14 cm diameter ^{31}P surface coil

167   (Philips Medical Systems, Best, the Netherlands) was strapped to the dominant quadriceps. A non-

168   ferromagnetic weight, equivalent to 10% of lean body mass, was strapped to the dominant ankle

169   (Figure 1).[17] This load was chosen to target an ~30% exertional fall in PCr from baseline thereby

170   avoiding any significant lowering of pH (<6.8) which prolongs PCr $t_{1/2}$.[21] Participants then practiced

171   knee extensions before full entry into the scanner bore, where they were positioned such that the centre

172   of the ^{31}P coil was at magnet isocenter. A series of triplanar ^{1}H scout images were acquired and used

173   for automatic shimming to optimize the B_0 field homogeneity. Continuous ^{31}P spectra were then

174   acquired (unlocalised, repetition time 2 sec, echo time 0.35 msec, bandwidth 2000 Hz, excitation flip

175   angle optimized for flip angle and 1024 data points) in conjunction with the exercise paradigm

176   consisting of 1 min rest, 1 min knee extensions at 0.5 Hz, and 5 min recovery. This was repeated to

177   permit two PCr $t_{1/2}$ measurements which were then averaged. The same MRS sequence was acquired

178   with a repetition time of 30 sec (8 averages) to provide a fully relaxed spectrum for the calculation of

179   metabolite concentrations.

180

181   ^{31}P MRS spectra were analysed using jMRUI v5.2 and quantified using the AMARES algorithm with

182   prior knowledge.[22,23] Absolute concentrations of PCr and P_i were calculated making the standard

183   assumption that resting ATP concentration is 8.2 mmol/L cytosolic water (i.e. mM).[24] Intramuscular

184   pH was estimated from the chemical shift of P_i using the following equation: pH = 6.75 + log (α-

185   3.27/5.69-α), where α is the chemical shift of the P_i peak relative to PCr.[25] Free cytosolic ADP

186   concentration was calculated by standard means using the creatine kinase equation: ADP = ATP x

187   creatine / [PCr x H^+ x 1.66 x 10^9 M^{-1}], and assuming that total creatine is 42.5 mM.[24] The PCr $t_{1/2}$ was

188   found by fitting the following monoexponential equation to the PCr recovery data: PCr(t) = $PCr_{initial}$ +

189   (PCr_{end} - $PCr_{initial}$)(1-e^{-kt}), where t is the time from the end of exercise, $PCr_{initial}$ and PCr_{end} are the PCr

190   concentrations at the start and end phase of recovery, and k is the recovery rate constant. PCr $t_{1/2}$ is

191   calculated as $log_e 2/k$. The analogous monoexponential equation was fitted to the ADP recovery data to

192   determine ADP $t_{1/2}$, another inverse marker of skeletal muscle energetics.[16]

193

194   **Secondary and Safety Endpoints**

195   Secondary endpoints included (1) skeletal muscle energetics as reflected by the ADP $t_{1/2}$ and the

196   intracellular concentrations of high-energy phosphate compounds and pH on dynamic ^{31}P MRS; (2) 6-

197   min walk distance; (3) peak oxygen consumption (VO_2) and the ratio of minute ventilation to CO_2

198 production on cardiopulmonary exercise testing; (4) symptoms as quantified by the NYHA class, Borg

199 dyspnea score, and fatigue scale (assessed using a 10-point visual analogue fatigue scale, from 1 = no

200 fatigue, to 10 = very severe fatigue); (5) quality of life as assessed by the KCCQ; (6) Hb and iron status

201 (ferritin, TSAT, soluble transferrin receptor); (7) N-terminal pro-B-type natriuretic peptide (NT-

202 proBNP); and (8) LVEF.

203

204 The 6-min walk test was performed using a flat, straight, 20-m corridor with turnaround points marked

205 by a chair at each end of the measured course. Patients were verbally encouraged to cover as much

206 ground at their own pace for 6 min and were asked to rate themselves on a Borg dyspnea scale before

207 and after exercising. The same blinded investigator supervised all 6-min walk tests for a specific

208 subject. Cardiopulmonary exercise testing was performed using a modified Naughton treadmill

209 protocol.[26] Maximal exercise capacity was attained if the respiratory exchange ratio was >1.00 or had

210 increased by ≥0.15 from the resting value. All treadmill exercise tests were supervised by blinded

211 cardiac physiologists.

212

213 Safety endpoints included (1) adverse events; (2) blood pressure; (3) heart rate; (4) respiratory rate; (5)

214 serum creatinine concentration; (6) serum AST; (7) serum C-reactive protein; and (8) blood pressure

215 and heart rate during treatment infusions.

216

217 **Statistics**

218 Statistical analyses were prespecified and followed the intention-to-treat principle. Sample size

219 estimates were hampered by a paucity of quadriceps ^{31}P MRS data in patients with CHF. Initial

220 calculations based on a trial using calf ^{31}P MRS,[27] suggested that 40 patients in total would be needed

221 to detect a 30 sec difference in PCr $t_{1/2}$ ($\alpha = 0.05$, $\beta = 90\%$, standard deviation = 24 sec) and cover for

222 drop-outs and missing data. Review of the accruing blinded data suggested that the variance and

223   standard deviation of PCr $t_{1/2}$ were likely to be smaller but that 40 patients in total was still sufficient to

224   detect a more conservative difference of 6 sec with a standard deviation of 5 sec ($\alpha = 0.05$, $\beta = 90\%$).

225

226   The primary analysis was a comparison of PCr $t_{1/2}$ at 2 weeks using an analysis of covariance model

227   with baseline PCr $t_{1/2}$ as covariate. All other continuous endpoints were similarly analysed. Missing

228   data were imputed using the last observation carry-forward method for patients who had a last

229   observation recorded post treatment. For those who did not, the mean value for their treatment group

230   was used for imputations. Sensitivity analyses without imputation and with a *post-hoc* Markov chain-

231   Monte Carlo imputation with 10 iterations were performed. Categorical variables were evaluated using

232   a Pearson's χ^2 test. Baseline and week-2 data are described using appropriate summary measures. The

233   estimates and two-sided 95% confidence intervals (95% CI) for the difference between least-squares

234   means of the two treatment arms are given. All statistical tests were two-sided and we judged a P-value

235   < 0.05 significant. All analyses were carried out using Stata 9.2 (Statacorp, Texas) and Statview 4.5 for

236   Windows (Abacus Concepts, California).

237

238   **RESULTS**

239   **Patients and Iron Isomaltoside Administration**

240   Between October 2014 and December 2016, we screened 83 outpatients (Figure 2) and randomized 40

241   to IIM (n=21) or placebo (n=19). Baseline characteristics were balanced between the two treatment

242   groups (Table 1). All patients received their allocated therapy at a single sitting except for 1 subject

243   who received placebo over 2 sittings. In those randomized to IIM, the mean iron repletion dose was

244   929±320 mg (11±4 mg/kg). No subject was lost to follow-up and only 1 patient did not attend an end-

245   of-study visit due to hospitalization. Overall, 4.2% of the analysed data was imputed. Sensitivity

246   analyses yielded consistent results.

247

248   **Primary Endpoint**

249   During dynamic ^{31}P MRS the treatment arms exercised against similar weights (IIM: 5.7±0.7 kg,

250   placebo: 5.5±0.9 kg, P=0.57) and achieved similar degrees of exertional PCr depletion at baseline (IIM:

251   36±11%, placebo: 34±13%, P=0.52) and at 2 weeks (IIM: 38±13%, placebo: 37±9%, P=0.71). At

252   baseline, post-exercise PCr $t_{1/2}$ was similar in the randomized groups (Table 1). After treatment, PCr $t_{1/2}$

253   improved (shortened) by 17% (-4±10 sec) in the IIM group and worsened by 7% (3±7 sec) in the

254   placebo arm (Table 2). The primary endpoint, PCr $t_{1/2}$ at 2 weeks, was significantly shorter in patients

255   randomized to IIM with an adjusted between-group difference of $-$ 6.8 sec (95% CI -11.5 to -2.1,

256   P=0.006; Table 2, Figure 3). This remained significant even after *post-hoc* adjustment for both baseline

257   PCr $t_{1/2}$ and Hb (-6.8 sec, 95% CI -11.6 to -2.0, P=0.007) or other variables that appeared unbalanced

258   such as age (-8.0 sec, 95% CI -12.8 to -3.2, P=0.002), and NTpro-BNP (-7.2 sec, 95% CI-10.5 to -1.0,

259   P=0.007). No anemia status by treatment group interaction existed (P=0.44).

260

261   **Secondary Endpoints**

262   *Skeletal Muscle ADP $t_{1/2}$, Metabolite Concentrations and pH*

263   Treatment with IIM significantly improved (shortened) post-exercise ADP $t_{1/2}$ (Table 2) by 45% (-5±9

264   sec) while it lengthened by 3% (3±7 sec) with placebo. No difference in resting or end-exercise PCr

265   and ADP levels were seen between the groups after treatment (Table 2). Neither the resting nor end-

266   exercise pH differed between the groups after treatment (Table 1 & 2), and only 1 patient at baseline,

267   and no patient at 2 weeks, had a pH ≤ 6.8 during exercise. Possibly due to this limited acidosis, no

268   correlation was seen between exertional pH decrements and the degree of PCr depletion, iron status or

269   the visual analogue fatigue scale. As expected, changes in ADP $t_{1/2}$ correlated with changes in PCr $t_{1/2}$

270   (r=0.65, P<0.001).

271

272   *Hemoglobin and Iron Status*

273   Ferritin increased by 83% (327±185 ng/mL) in the IIM group and decreased by 24% (2±27 ng/mL) in

274   the placebo group (Table 2). Similarly, TSAT increased by 29% (8±6%) with IIM and by 4% (2±9%)

275   with placebo. Despite this, Hb remained largely unchanged, being minimally increased by 0.4% (0.6±9

276   g/L) in the IIM arm, and minimally decreased by 0.8% (-1±14 g/L) in the placebo arm. Changes in

277   ferritin (log transformed; r= -0.37, P=0.02) but not Hb (r=0.17, P=0.30) correlated with changes in PCr

278    $t_{1/2}$.

279

280   *Symptoms, Exercise Capacity, LVEF and NT-proBNP*

281   Therapy with IIM improved symptoms as reflected by reductions in NYHA class and the post-exercise

282   Borg dyspnea score (Table 2). After 2 weeks, 2 (10%), 19 (90%), and 0 (0%) patients in the IIM group

283   had an improved, unchanged, or worse NYHA class, respectively. By contrast, 1 (5%), 15 (79%), and 3

284   (16%) patients in the placebo group had an improved, unchanged, or worse NYHA class. There was no

285   difference in the pre-exercise Borg dyspnea score (-0.6, 95% CI -1.5 to 0.2, P=0.12), visual analogue

286   fatigue scale or the KCCQ score (Table 1). Symptomatic improvements were not accompanied by

287   significant changes in LVEF, NT-proBNP, 6-min walk distance, visual analogue fatigue scale (-0.59,

288   95% CI -1.45 to 0.28, P=0.18), peak VO_2 or the ratio of minute ventilation to CO_2 production (-0.2,

289   95% CI -3.3 to 2.9, P=0.88) despite both groups attaining similar peak respiratory exchange ratios at

290   baseline (Table 1) and at week 2 (IIM: 1.10±0.10, placebo: 1.05±0.09). Changes in 6-min walk

291   distance related to changes in PCr $t_{1/2}$ (r= -0.33, P=0.04), and peak VO_2 related to PCr $t_{1/2}$ in the IIM

292   (r=-0.57, P=0.007) but not placebo (r=-0.30, P=0.21) group at 2 weeks.

293

294   **Safety Endpoints**

295   The incidence of adverse events was comparable between the treatment arms with 3 (14%) and 1 (5%)

296   events in the IIM and placebo groups, respectively (P=0.34). In the IIM arm, 1 patient had arthralgia

297   during the infusion, 1 patient noted a mild rash at the venepuncture site 1 day post-infusion, and 1

298   patient had a serious adverse event (hospitalized 1 week post-infusion with unstable angina needing

299   coronary artery bypass surgery) that was judged unrelated to study drug. No anaphylactic reactions

300   occurred. In the placebo arm, 1 patient reported coryzal symptoms 3 days post-infusion.

301

302   Infusions of IIM had no impact on hemodynamics. Systolic blood pressure remained unchanged from

303   pre-infusion (122±17 mmHg) to 15 (123±17 mmHg), 30 (122±17 mmHg), 45 (119±19 mmHg), and 60

304   (121±15 mmHg) minutes post-infusion (all P>0.05). Diastolic blood pressure and heart rate were

305   similarly stable. At 2 weeks, no differences between the treatment arms were seen for blood pressure,

306   heart rate, and serum creatinine, AST or CRP levels, but IIM was associated with significantly reduced

307   respiratory rates (Table 2).

308

309   **Subgroup Analysis**

310   Baseline characteristics for the anemic and non-anemic subgroups stratified by treatment allocation are

311   shown in Table 1. Mean iron repletion dose was 1155±221 mg (13±2 mg/kg) in anemic patients and

312   680±204 mg (9±3 mg/kg) in non-anemic patients. Baseline PCr $t_{1/2}$ was similar in the randomized

313   groups in both anemic and non-anemic subpopulations. Infusion of IIM significantly improved

314   (shortened) PCr $t_{1/2}$ at 2 weeks in anemic patients. A smaller improvement in non-anemic subjects did

315   not reach statistical significance (Table 3).

316

317

318 **DISCUSSSION**

319 FERRIC-HF II sought to illuminate how iron repletion could dramatically augment exercise

320 performance in CHF despite minimal Hb changes. We found that, in iron-deficient patients with CHF

321 and an LVEF ≤45%, a single total dose infusion of IIM safely repleted iron stores and was associated

322 with faster skeletal muscle post-exercise PCr recovery kinetics at 2 weeks, implying better

323 mitochondrial function. Enhancements in skeletal muscle energetics occurred despite no change in Hb,

324 but were paralleled by improvements in symptoms as reflected by reductions in NYHA class and the

325 post-exercise Borg dyspnea score. Augmented skeletal muscle energetics might therefore be an

326 important mechanism via which iron repletion improves functional capacity despite eliciting minimal

327 Hb changes.

328

329 Dynamic ^{31}P MRS is uniquely able to quantify *in vivo* skeletal muscle energetics non-invasively. At

330 rest, skeletal muscle pH and phosphometabolite (PCr, Pi, ADP) concentrations are dictated not by ATP

331 supply and demand but by sarcolemmal Na^+-dependent Pi and creatine uptake, Na^+/H^+-antiporter

332 activity, and the net balance of adenine nucleotide breakdown and synthesis.[16] At the onset of exercise,

333 ATP consumption by myosin-ATPases increases ATP demand which is met immediately by local PCr

334 breakdown via the creatinine kinase reaction to regenerate ATP.[7] Sustained exertion is then driven by

335 mitochondrial PCr regeneration via OXPHOS and glycolysis.[5-8] Therefore, during exertion, ATP levels

336 remain largely unchanged due to rapid PCr buffering, PCr declines due to its heightened consumption,

337 pH falls due to elevated glycolysis, and ADP and Pi rise due to increased ATP hydrolysis and PCr

338 consumption. At the end of exercise, PCr consumption and glycolysis cease and PCr levels start to

339 recover at a rate commensurate with mitochondrial oxidative ATP synthesis.[24] Dynamic ^{31}P MRS

340 enables the real-time noninvasive tracking of these biochemical events *in situ*, with ^{31}P MRS measures

341 closely related to peak VO$_2$ and invasive markers of muscle energetics.[16,28,29] In our cohort, resting pH

342 and phosphometabolite concentrations mirrored those reported in other CHF patients and normal

343   subjects[30-32] and were unaltered by IIM. This implies that the processes dictating resting skeletal

344   muscle indices were unperturbed in our subjects and were not influenced by IIM over 2 weeks. On

345   initiation of mild to moderate exercise in-magnet, skeletal muscle ATP levels remained constant, PCr

346   and pH fell, and ADP and Pi rose in our patients as expected (see Figure 2). That the degree of

347   exertional PCr depletion was similar in the treatment groups implies comparable effort. That only 1

348   patient developed significant intracellular acidosis (exercise pH <6.8), which slows PCr $t_{1/2}$,[21] implies

349   that our exercise paradigm did not evoke significant glycolysis and that our PCr $t_{1/2}$ values likely truly

350   reflected oxidative ATP synthesis capacity.

351

352   To the best of our knowledge, this is the first study to show that iron repletion with IIM augments

353   skeletal muscle energetics as reflected by a 14% relative acceleration of PCr recovery kinetics on

354   dynamic ^{31}P MRS. This represents a substantial benefit. In a randomized trial in CHF patients,

355   Adamapolous et al., reported a 5% weekly rate of improvement in PCr $t_{1/2}$ with moderate-intensity

356   exercise training.[33] In a double-blind evaluation of the energetic enhancer perhexiline, Lee et al.,

357   documented a 4% weekly rate of improvement in PCr $t_{1/2}$ in CHF patients.[27] In young healthy

358   individuals, 2 weeks of high-intensity exercise training improved PCr recovery by 14% with no change

359   in the untrained control group.[34] Thus, over 2 weeks, a single total repletion dose of IIM augmented

360   skeletal muscle energetics to the same extent as 3 weeks of moderate-intensity exercise training in

361   CHF, 3-4 weeks of perhexiline use in CHF, and 2 weeks of high-intensity physical training in younger

362   healthy individuals. In contrast, Melenovsky et al., very recently found no difference in PCr recovery

363   kinetics 4 weeks after infusing Ferric Carboxymaltose (1g) in 13 CHF patients.[35] This might be because

364   their patients exercised longer (6 mins vs. 1 min) against higher resistance (7kg vs. 5.6±0.8kg) during

365   ^{31}P MRS, leading to greater intracellular acidosis (pH 6.92±0.17 vs 6.98±0.07) which renders PCr

366   recovery kinetics less informative of OXPHOS.[16,18,21] This might also reflect their smaller sample size,

367   lack of randomization and a control group, later reassessment at 4 weeks, or the use of Ferric

368   Carboxymaltose which uniquely induces hypophosphatemia which could blunt energetic gains from

369   iron repletion.[36-38] Several explanations for our findings can be posited.

370

371   Post-exercise PCr $t_{1/2}$ is influenced by skeletal muscle O_2 supply (determined by cardiopulmonary

372   function and Hb), storage (myoglobin), and utilization (mitochondria),[16] which are all modifiable by

373   iron. While we had no pulmonary data, cardiac function and hemodynamics were unaltered by IIM

374   consistent with evidence that O_2 delivery does not limit post-exercise PCr recovery in CHF.[39] Skeletal

375   muscle myoglobin levels are also reportedly normal and exertional myoglobin desaturation in CHF

376   does not restrict skeletal muscle metabolism.[40,41] So, the benefits of IIM are unlikely mediated via O_2

377   storage. A key mechanistic finding in FERRIC-HF II was our observation that Hb levels did not change

378   with IIM. This accords with data in CHF showing that ID impairs exercise tolerance independently of

379   Hb,[1,2] that iron repletion improves functional capacity despite minimal Hb changes,[3,4] and that such Hb

380   changes do not correlate with increments in exercise performance.[3,4] Although skeletal muscle benefits

381   were greater in our anemic subgroup, this is probably not a function of lower Hb *per se* but may reflect

382   the fact that anemic patients had a poorer iron status than non-anaemics. Thus, IIM likely improved

383   energetics by altering skeletal muscle O_2 utilization.

384

385   Mitochondria power cellular processes and mitochondrial dysfunction due to ID might have been

386   mitigated by IIM via numerous mechanisms. This includes acceleration of the electron transport chain

387   which sets the pace for OXPHOS and contains iron-sulfur cluster and heme prosthetic groups.[8,12] Iron

388   is also embedded in enzymes of the Krebs cycle, fatty acid ß-oxidation, and carnitine synthesis,[42] and is

389   active in catalase which maintains a permissive redox environment for efficient OXPHOS activity.[43] In

390   human CHF, myocardial ID is linked to diminished catalase and Krebs cycle enzyme levels.[44] In *in*

391   *vitro* and animal studies, cellular ID impairs cardiomyocyte and skeletal muscle energetics even in the

392   absence of anemia.[13,14,45] Indeed, in rats with ID anemia, correction of anemia but not ID failed to

393 improve submaximal exercise capacity (which is determined by oxidative enzymes).[15] In contrast,

394 correction of ID but not anemia improved submaximal exercise performance within 15 hours of iron

395 infusions.[46]

396

397 Besides skeletal muscle energetic augmentation by IIM, several other aspects of our study bear special

398 emphasis. First, symptoms were improved by IIM at 2 weeks, with reductions in NYHA class and Borg

399 score corroborated by attenuations in resting respiratory rate (presumably due to improved respiratory

400 muscle energetics). Apart from diuretics, no other contemporary CHF therapy can likely match this

401 pace of symptomatic benefit. Second, this is the first trial to test the utility of infusing a total repletion

402 dose of iron at a single sitting. By 2 weeks, we achieved the same degree of biochemical iron repletion

403 as was achieved by 6 to 12 months in prior studies.[4] Third, FERRIC-HF II is the first to show, in a

404 double-blind placebo-controlled trial, that IIM ameliorates ID in CHF patients.

405

406 Our study has limitations. We were underpowered to detect differences in certain clinical indices (e.g.,

407 peak VO$_2$ and 6-min walk distance), in safety endpoints such as rare side-effects of IIM, and in the

408 anemic and non-anemic subgroups. This might explain some of our modest correlations and the near-

409 significant effect of IIM on PCr $t_{1/2}$ in non-anemics which merits further study. We did not measure

410 skeletal muscle blood flow so cannot exclude improved limb perfusion as a contributor to enhanced

411 muscle energetics. However, blood supply does not limit skeletal muscle metabolism in CHF,[39] so it is

412 unlikely that any theoretical increase in perfusion with IIM could influence PCr $t_{1/2}$. We chose a short

413 trial duration to minimize attribution of results to skeletal muscle exercise adaptation, so the energizing

414 effect of IIM might have been greater with a longer trial. No adjustments were made for multiple

415 comparisons and we used the last observation carry forward method for imputations.

416

417   FERRIC-HF II has potentially important clinical ramifications. Despite data that ID is common and

418   adverse in CHF,[1,2] and that its correction confers benefits,[3,4] clinical uptake of routine iron repletion,

419   particularly in non-anemic patients, remains poor. One potential barrier to adoption is the lack of

420   mechanistic data explaining how iron could trigger such profound exercise benefits despite minimal

421   changes in Hb. Our findings should therefore go some way to reassure clinicians that the benefits of IV

422   iron are real and appear to have a sound mechanistic underpinning. Second, our data reinforces the

423   importance of routinely checking iron indices in CHF patients, and emphasizes that the treatment target

424   should be iron status and not necessarily Hb. Third, our study suggests that a single total repletion dose

425   of IIM can safely replete iron stores with clinical benefits.

426

427   In conclusion, the FERRIC-HF II trial has shown that, in patients with CHF and ID, a total repletion

428   dose of IIM given at a single sitting is well tolerated and associated with faster skeletal muscle post-

429   exercise PCr recovery kinetics at 2 weeks, implying better mitochondrial function. Enhanced skeletal

430   muscle energetics occurs despite no change in Hb, but is accompanied by improved symptoms.

431   Augmented skeletal muscle energetics might therefore be an important mechanism via which iron

432   repletion improves exercise capacity in CHF despite minimal changes in Hb.

433

452 **DISCLOSURES**

453 Dr Okonko has received speakers fees and research support from Pharmacosmos, and speakers fees

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456

457

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	All patients		Anemic patients		Nonanemic patients	
	Placebo (n=19)	Iron Isomaltoside (n=21)	Placebo (n=9)	Iron Isomaltoside (n=11)	Placebo (n=10)	Iron Isomaltoside (n=10)
Demographics						
Age, years	62±13	70±12	63±15	74±6	61±11	65±15
Male gender, n (%)	13(68)	16(76)	7(78)	11(100)	6(60)	5(50)
Caucasian ethnicity, n (%)	14(74)	17(81)	6(67)	10(91)	8(80)	7(70)
Body mass index, kg/m^2	30±7	29±4	29±6	29±4	30±8	29±5
Ischemic etiology, n (%)	10(53)	11(52)	6(67)	7(64)	4(40)	4(40)
Comorbidities						
Coronary artery disease	11(58)	13(62)	6(67)	9(82)	5(50)	4(40)
Hypertension	13(64)	13(62)	5(56)	8(73)	8(80)	5(50)
Hyperlipidemia	7(37)	7(33)	2(22)	6(55)	5(50)	1(10)
Diabetes mellitus	10(53)	10(48)	6(67)	7(64)	4(40)	3(30)
Atrial fibrillation/flutter	4(21)	6(29)	2(22)	3(27)	2(20)	3(30)
Clinical and quality of life						
LV ejection fraction, %	37±8	37±8	37±8	36±9	37±7	39±7
NYHA class	2.4±0.5	2.5±0.5	2.5±0.5	2.4±0.5	2.5±0.5	2.4±0.5
NYHA class III, n (%)	10(53)	9(43)	4(44)	6(55)	6(60)	3(30)
Systolic BP, mm Hg	122±17	124±16	115±14	125±14	128±17	124±18
Diastolic BP, mm Hg	71±14	73±10	67±11	71±8	75±16	76±12
Heart rate, beats/min	71±11	72±10	67±6	71±6	74±13	72±13
Respiratory rate, breaths/min	16±1	16±1	16±1	16±1	16±1	15±1
KCCQ overall score	53±20	64±23	58±27	66±19	48±13	62±28
Exercise parameters						
Peak VO$_2$, mL/kg/min	14.3±3.1	15.8±4.3	12.7±3.2	16.0±5.6	15.8±2.2	15.6±2.5
Peak respiratory exchange ratio	1.06±0.11	1.10±0.10	1.05±0.10	1.14±0.10	1.07±0.12	1.07±0.10
CPET exercise duration, s	582±202	627±241	551±252	636±289	610±153	617±191
6 min walking distance, m	313±67	324±79	295±70	299±84	330±64	351±68
Pre-exercise Borg dyspnea score	11±1	11±1	11±1	11±2	11±1	11±1
Post-exercise Borg dyspnea score	15±2	14±3	15±2	15±3	14±2	14±2

Table 1. Baseline characteristics. Data are mean±SD, numbers(%), or median (interquartile range).

	All patients		Anemic patients		Nonanemic patients	
	Placebo (n=19)	Iron Isomaltoside (n=21)	Placebo (n=9)	Iron Isomaltoside (n=11)	Placebo (n=10)	Iron Isomaltoside (n=10)
^{31}P MRS measurements						
Resting PCr, mM	39±4	40±6	39±4	42±7	40±5	38±5
Exercise PCr, mM	26±4	25±6	23±5	25±7	28±3	25±5
Resting Pi, mM	4.3±1.0	4.0±0.7	4.4±1.0	3.9±0.8	4.1±1.1	4.2±0.6
Exercise Pi, mM	18.3±6.8	19.1±7.2	18.8±6.9	20.7±8.8	17.9±7.1	17.3±4.8
Resting pH	7.01±0.04	7.02±0.03	7.01±0.02	7.02±0.02	7.01±0.06	7.01±0.03
Exercise pH	7.00±0.05	6.96±0.10	7.00±0.04	6.97±0.07	7.00±0.06	6.95±0.13
Resting ADP, µM	8±5	8±6	8±6	7±4	8±5	10±8
Exercise ADP, µM	34±12	38±21	40±15	40±26	28±5	35±15
ADP $t_{1/2}$, s	23±6	25±9	24±6	26±12	23±6	24±4
PCr $t_{1/2}$, s	33±9	35±12	36±8	38±14	29±8	31±9
Laboratory measurements						
Ferritin, ng/mL	59(39-79)	34(18-50)	45(26-64)	33(18-48)	77(64-90)	44(28-60)
Transferrin saturation, %	18±10	21±8	12±5	16±7	24±10	25±6
Soluble transferrin receptor, mg/L	4.0±1.5	3.6±0.8	4.6±1.5	3.9±0.6	3.4±1.3	3.4±0.9
Hemoglobin, g/L	128±20	130±15	114±19	119±8	140±11	142±10
Creatinine, µmol/L	108±34	121±39	129±32	138±21	89±24	103±46
Aspartate transaminase, iU/L	22±9	22±8	25±8	21±7	20±10	24±8
C-reactive protein, mg/L	5(1-10)	6(3-9)	12(2-22)	5(3-8)	3(1-6)	6(1-11)
NT-proBNP, pg/mL	462(206-855)	1486(245-2054)	790(291-1944)	2696(423-3907)	316(133-629)	696(245-1900)
Treatment						
Diuretics, n (%)	12(63)	14(67)	8(89)	7(64)	4(40)	7(70)
ACE-inhibitor or ARB, n (%)	17(89)	16(76)	7(78)	9(82)	10(100)	7(70)
Beta-blockers, n (%)	16(84)	18(86)	7(78)	10(91)	9(90)	8(80)
Spironolactone, n (%)	12(63)	12(57)	5(56)	5(45)	7(70)	7(70)
Digoxin, n (%)	4(21)	6(29)	3(33)	2(18)	1(10)	4(40)
Anticoagulants, n (%)	3(16)	6(29)	1(11)	3(27)	2(20)	3(30)
Antiplatelets, n (%)	13(68)	13(62)	5(56)	8(73)	8(80)	5(50)

Table 1. Baseline characteristics (continued). Data are mean±SD, numbers (%), or median (interquartile range).

	Placebo (n=19)	Iron Isomaltoside (n=21)	Difference (95% CI)	ANCOVA P-value
Primary endpoint				
PCr $t_{1/2}$, s	36±11	30±7	-6.8(-11.5,-2.1)	**0.006**
Secondary endpoints				
ADP $t_{1/2}$, s	24±9	20±6	-5.3(-9.7,-0.9)	**0.02**
Hemoglobin, g/L	127±14	130±13	2.4(-3.5,8.4)	0.41
Ferritin, ng/mL	57(41-84)	369(232-495)	304(217,391)	**<0.0001**
Transferrin saturation, %	21±9	29±6	6.8(2.7,10.8)	**0.002**
NYHA class	2.6±0.5	2.3±0.5	-0.23(-0.46,-0.01)	**0.04**
6 min walking distance, m	324±67	347±72	15(-10,40)	0.24
Pre-exercise Borg dyspnea score	8±2	7±1	-0.6(-1.5,0.2)	0.12
Post-exercise Borg dyspnea score	15±2	12±3	-2.0(-3.7,-0.3)	**0.02**
Peak VO_2/kg, mL/kg/min	14.9±3.5	16.8±4.7	0.5(-1.0,1.9)	0.54
KCCQ overall score	55±24	68±17	12.7(-7.7,33.2)	0.18
LV ejection fraction, %	39±8	41±7	2.2(-1.1,5.6)	0.19
NT-proBNP, pg/mL	334(180-827)	1623(281-2453)	289(-461,1040)	0.44
Resting PCr, mM	40±5	40±6	-0.2(-3.5,3.0)	0.89
Exercise PCr, mM	26±6	25±6	-0.3(-3.6,3.0)	0.86
Resting Pi, mM	3.8±1.1	4.0±1.0	0.3(-0.3,0.8)	0.33
Exercise Pi, mM	18.2±7.2	17.6±6.7	-0.9(-5.0,3.2)	0.66
Resting pH	7.01±0.3	7.01±0.3	-0.01(-0.02,0.01)	0.44
Exercise pH	6.99±0.06	6.97±0.07	0(-0.04,0.04)	0.97
Resting ADP, μM	7±5	8±5	0.9(-2.0,3.8)	0.52
Exercise ADP, μM	34±15	37±18	1.2(-8.1,10.5)	0.80
Safety endpoints				
Systolic blood pressure, mm Hg	119±14	127±12	7.1(-0.3,14.5)	0.06
Diastolic blood pressure, mm Hg	72±13	74±10	0.7(-5.5,6.8)	0.83
Heart rate, beats/min	74±12	71±10	-2.9(-8.8,3.1)	0.34
Respiratory rate, breaths/min	16±1	15±1	-0.7(-1.2,-0.2)	**0.009**
Creatinine, μmol/L	103±38	106±41	-3(-26,20)	0.80
Aspartate transaminase, iU/L	22±6	30±26	8(-4,21)	0.21
C-reactive protein, mg/L	4(2-8)	6(2-11)	0.4(-3.0,3.9)	0.79

Table 2. Endpoints for total population

	Placebo	Iron Isomaltoside	Difference (95% CI)	ANCOVA P-value
Anemic patients	*n=9*	*n=11*		
PCr $t_{1/2}$, s	38±12	30±9	-8.4(-16.7,-0.2)	**0.04**
ADP $t_{1/2}$, s	24±9	18±7	-6.3(-13.5,0.9)	0.08
Hemoglobin, g/dL	117±12	124±6	5.5(-2.7,13.8)	0.18
Ferritin, ng/mL	45±25	456±161	413(295,530)	**<0.0001**
NYHA class	2.7±0.5	2.5±0.5	-0.3(-0.6,0.1)	0.09
Non-anemic patients	*n=10*	*n=10*		
PCr $t_{1/2}$, s	35±11	31±5	-5.2(-10.6,0.2)	0.06
ADP $t_{1/2}$, s	24±9	21±5	-4.4(-9.6,0.9)	0.10
Hemoglobin, g/dL	135±9	137±15	0.5(-8.3,9.3)	0.72
Ferritin, ng/mL	67±24	274±149	176(68,284)	**0.003**
NYHA class	2.6±0.5	2.2±0.4	-0.2(-0.6,0.1)	0.23

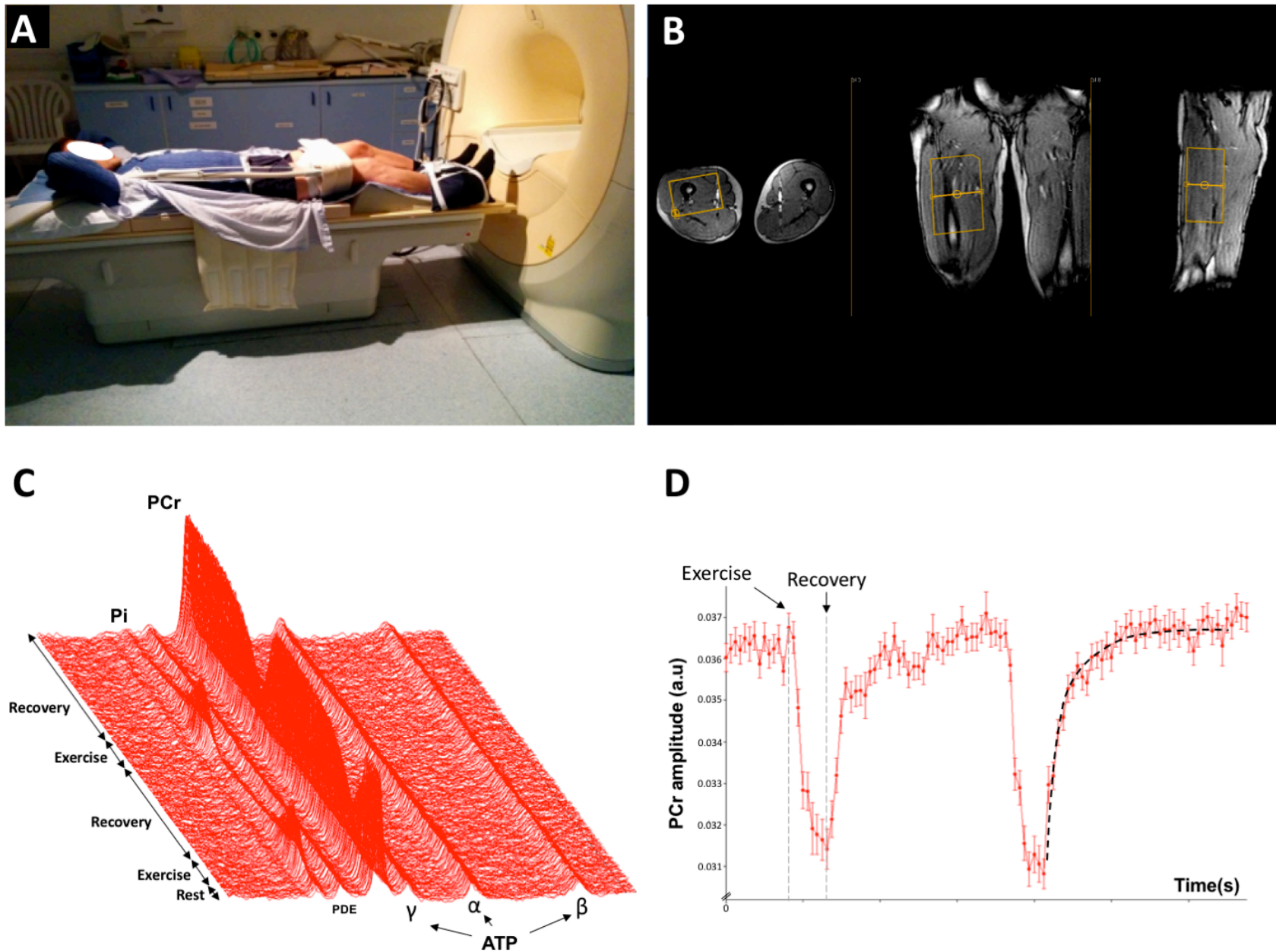**Table 3. Endpoints for anemic and non-anemic subgroups**

Fig. 1. Dynamic Quadriceps ^{31}P Magnetic Resonance Spectroscopy (^{31}P MRS). The figure shows the experimental setup and example data from multiple studies. Experiments are performed with subjects supine (A) and ^{31}P coil and leg weight attached prior to entry into the magnet bore. ^{1}H scout images of quadriceps are used to optimize positioning (B). ^{31}P MRS data are shown as stacked plot of all phosphometabolite changes (C) and a timecourse of phosphocreatine signal (D) acquired during two bouts of exercise and recovery; PCr $t_{1/2}$ is calculated from the recovery curve (interrupted line) shown in D. PDE = phosphodiester.
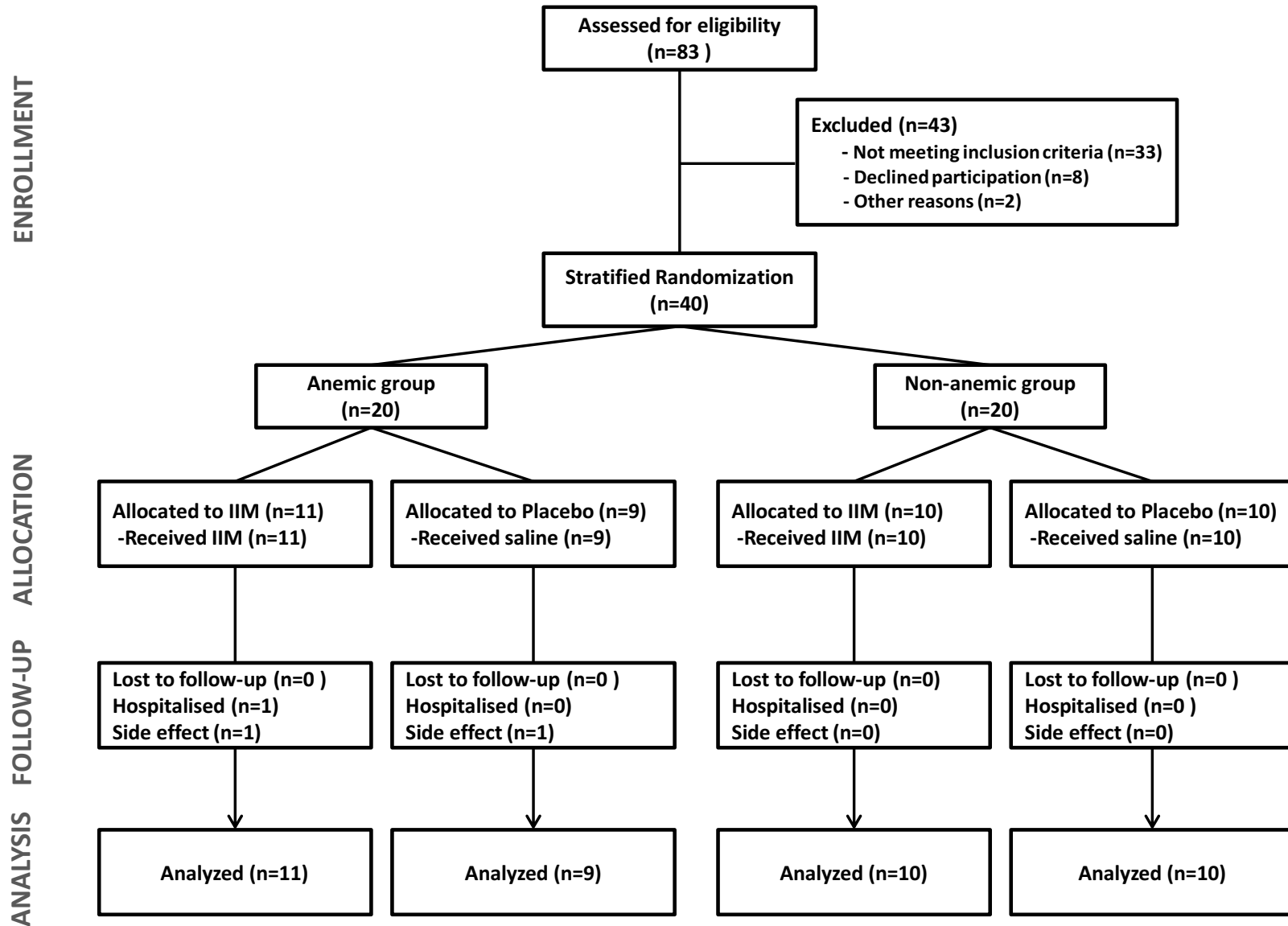
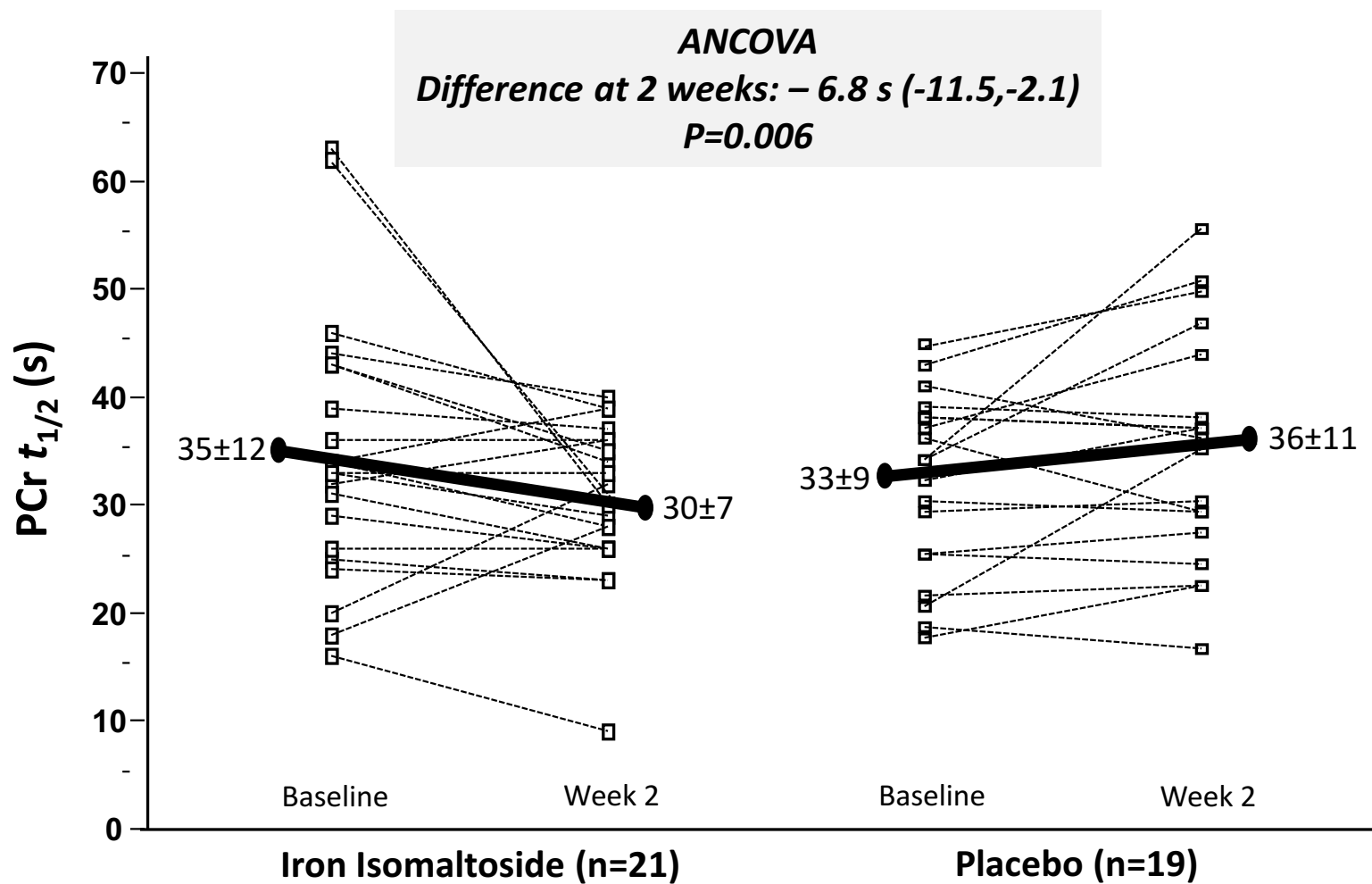**Fig. 2. FERRIC-HF II CONSORT Diagram.** Patient disposition during the trial.

Fig. 3. Primary Endpoint. Individual changes in PCr $t_{1/2}$ with Iron Isomaltoside and saline placebo in total cohort.