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2 **Effects of aerobic interval training and continuous training on cellular markers of**
3 **endothelial integrity in coronary artery disease: A SAINTEX-CAD substudy.**

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19 **Running title:** Effect of exercise training on EPC, angiogenic T cells and EMP in CAD

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26

27 **ABSTRACT**

28

29 **Background:** In this large multicenter trial, we aimed to assess the effect of aerobic exercise
30 training in stable coronary artery disease (CAD) patients on cellular markers of endothelial
31 integrity, and examine their relation with improvement of endothelial function.

32 **Methods:** Two-hundred CAD patients (LVEF >40%, 90% male, mean age 58.4±9.1 years)
33 were randomized on a 1:1 base to a supervised 12-week rehabilitation program of either
34 aerobic interval training (AIT) or aerobic continuous training (ACT) on a bicycle. At baseline
35 and after 12 weeks, numbers of circulating CD34+/KDR+/CD45dim endothelial progenitor
36 cells (EPC), CD31+/CD3+/CD184+ angiogenic T-cells and CD31+/CD42b- endothelial
37 microparticles (EMP) were analyzed by flow cytometry. Endothelial function was assessed by
38 flow-mediated dilation (FMD) of the brachial artery.

39 **Results:** After 12 weeks of AIT or ACT, numbers of circulating EPC, angiogenic T-cells and
40 EMP were comparable to baseline levels. Whereas improvement in peak VO₂ was correlated
41 to improvement in FMD (pearson $r = 0.17$, $p = 0.035$), a direct correlation of baseline or post-
42 training EPC, angiogenic T-cells and EMP levels with FMD was absent. Baseline EMP related
43 inversely to the magnitude of the increases in peak VO₂ (spearman $\rho = -0.245$, $p = 0.027$)
44 and FMD (spearman $\rho = -0.374$, $p = 0.001$) following exercise training.

45 **Conclusions.** Endothelial function improvement in response to exercise training in CAD
46 patients is not mediated by increased releases of EPC and angiogenic T-cells and/or a
47 diminished shedding of EMP into the circulation. EMP flow cytometry may be predictive of
48 the increases in aerobic capacity and endothelial function.

49

50 **Keywords:** Exercise training- Endothelial progenitor cells- endothelial microparticles-
51 coronary artery disease

52

53 INTRODUCTION

54

55 Exercise training is recognized as an important preventive and therapeutic strategy in
56 cardiovascular disease, in part through its beneficial effects on aerobic capacity and
57 endothelial function.(19) The strong and independent prognostic role of reduced physical
58 fitness and endothelial dysfunction has been demonstrated in healthy populations, as well
59 as in patients with underlying coronary artery disease (CAD) and heart failure. Small single
60 center studies show that peak oxygen uptake (peak VO_2) increases to a larger extent with
61 aerobic interval training (AIT) at higher intensity compared to moderate intensity continuous
62 training in patients with metabolic syndrome, after acute coronary syndrome, following
63 coronary artery bypass surgery and in patients with ischemic heart failure.(18, 20, 31, 32)
64 Interestingly, in the latter patient group, as well as in patients with metabolic syndrome, AIT
65 also led to a significantly larger improvement in peripheral endothelial function.(25, 32) The
66 reason for the superior effect of AIT on endothelial function is not completely understood. It
67 is, however, conceivable that differences in vascular shear stress patterns between exercise
68 protocols induce different molecular and cellular responses.

69 Indeed, the enhanced production of nitric oxide (NO) in response to shear stress plays a
70 critical role in the beneficial effects of exercise training on endothelial function. In addition,
71 the anti-inflammatory, free radical reducing and permeability decreasing properties of
72 exercise may all contribute to improvement of endothelial function.(10)

73 It has also been suggested that endothelial progenitor cells (EPC) could add to these
74 favorable changes.(16) EPC are a rather small population of cells that can be mobilized from
75 the bone marrow into the peripheral blood by various stimuli, such as ischemia and
76 chemokines. They participate in the repair of endothelial damage, thereby possibly
77 influencing endothelial function.(16) EPC function is regulated by angiogenic T-cells that
78 express the platelet endothelial cell adhesion molecule (CD31) and the receptor for stromal-

79 derived factor 1 (CD184).(14) Thus far, because of small-scale, single-center trials,
80 heterogeneity in patient populations and inconsistency of study results, it was not possible
81 to draw firm conclusions on a role for EPC and angiogenic T-cells as mediators of the
82 training-induced improvement of endothelial function in CAD.(8)

83 The main objective of the Study on Aerobic INTerval EXercise training in CAD (SAINTEX-CAD)
84 study was to investigate whether a 12-week program of AIT yields a larger gain in peak VO₂
85 and endothelial function compared to a similar training program of aerobic continuous
86 training (ACT).(6) The results of this large randomized multicenter study involving 200 CAD
87 patients demonstrated that AIT and ACT are equal in improving peak VO₂ and peripheral
88 endothelial function.(5) The purpose of this substudy from SAINTEX-CAD is to investigate if
89 aerobic exercise training (AIT versus ACT) can mobilize EPC and other related cellular blood
90 markers of endothelial integrity, and to examine the relationship between these blood
91 markers and the improvement of endothelial function.

92

93 **METHODS**

94

95 ***Patients and study design***

96 A detailed description of the rationale and design of the SAINTEX-CAD study has been
97 published previously.(6) Briefly, 200 stable patients with cardiovascular disease were
98 enrolled at the Cardiac Rehabilitation centers of the University Hospitals of Antwerp (Center
99 1, n=100) and Leuven (Center 2, n=100), Belgium, between October 2011 and April 2013.

100 The main study inclusion criteria were: 1) angiographically documented CAD or previous
101 acute myocardial infarction (AMI), 2) left ventricular ejection fraction (LVEF) > 40%, 3)
102 optimal medical treatment, 4) stable with regard to symptoms and medication for at least 4
103 weeks and 5) included between 4 and 12 weeks following AMI, elective percutaneous
104 coronary Intervention (PCI) or coronary artery bypass grafting (CABG). Patients were

105 randomized to either aerobic interval training (AIT) or aerobic continuous training (ACT) on a
106 1:1 base by an online protocol at Center 1. At baseline and after 3 months, patients
107 underwent cardiopulmonary exercise testing, vascular function assessment and blood
108 sampling. The Laboratory of Cellular and Molecular Cardiology of Center 1 served as the
109 central core laboratory responsible for the EPC and angiogenic T-cell analyses of both
110 centers.

111 The study complies with the Declaration of Helsinki, was approved by the local ethics
112 committees and written informed consent was obtained from each participant.

113

114 ***Exercise training***

115 Thirty-six supervised exercise sessions were implemented at a rate of 3 sessions a week
116 during 12 weeks. Patients exercised on a bicycle; exercise load was adjusted in order to
117 comply with the target heart rate (HR) throughout the 12-week training period. Patients
118 randomized to the AIT group cycled during 38 min in four 4-min intervals at 90–95% of peak
119 HR (Figure 1). Each interval was separated by 3-min active pauses, cycling at 50–70% of peak
120 HR. The session started with 10 min warm up and ended with a 3 min cool-down. Patients in
121 the ACT group cycled continuously at an intensity of at least 70-75% of peak HR during 37
122 min. The session started with 5 min warm-up and ended with a 5 min cool-down.

123

124 ***Clinical assessments***

125 Cardiopulmonary exercise testing

126 Cardiopulmonary exercise testing (CPET) was performed at baseline and after 12 weeks
127 using an individualized cycle ergometer ramp protocol (20 Watt +20 Watt/min or 10 Watt +
128 10 Watt/min). Breath-by-breath gas exchange measurements allowed on-line determination
129 of ventilation (VE), oxygen uptake (VO₂) and carbon dioxide production (VCO₂) every 10 s.
130 Peak VO₂ was determined as the mean value of three measures of VO₂ during the final 30 s

131 of exercise. The anaerobic threshold (AT) and the respiratory exchange ratio (RER) were
132 recorded.

133

134 Endothelium-dependent vasodilation

135 All analyses were performed in the morning, in fasting conditions and in a quiet
136 temperature-controlled room (21-24°C) by a trained operator that was blinded for the study
137 intervention. Subjects refrained from exercise, food and caffeine at least 8 hours before the
138 measurements. Blood pressure was obtained after 10 minutes of rest with an automated
139 blood pressure monitor. Endothelial function was assessed by flow-mediated dilation (FMD)
140 of the brachial artery using ultrasound (Center 1, AU5 Ultrasound System, Esaote; Center 2,
141 GE Healthcare, Vivid 7), according to the International Brachial Artery Reactivity Task Force
142 guidelines.⁽⁷⁾ A high-resolution linear-array vascular probe was used (Center 1, 10 MHz;
143 Center 2, 5-13 MHz). Patients were positioned supine with the right arm resting on an arm
144 support; the brachial artery was imaged above the antecubital fossa in the longitudinal
145 plane. After recording of the baseline diameter for at least 1 minute of stable distension
146 waveforms, a blood pressure cuff at the forearm was inflated to at least 200 mmHg or 60
147 mmHg higher than the resting systolic blood pressure. After cuff deflation, images were
148 recorded for 3 minutes. Images were analyzed using edge-detection software FMD-i by
149 Flomedi (Flomedi, Brussels, Belgium). FMD was expressed as the change in post-stimulus
150 diameter as a percentage of the baseline diameter. Analyses were blinded in both study
151 centers.

152

153 ***Flowcytometric quantification of cellular markers of endothelial integrity***

154 Quantification of EPC

155 EPC were defined as CD34+KDR+CD45dim cells.⁽²³⁾ Fixated whole blood (TransFix, Caltag
156 Medsystems, Buckingham, UK) was processed 2 to 3 days after sampling.⁽¹³⁾ Red blood cells

157 were lysed using ammonium chloride solution (NH₄Cl). After pre-treatment with Fc receptor
158 blocking reagent (Miltenyi Biotec, Bergisch Gladbach, Germany), samples were stained with
159 the following antibodies: CD34-PE-Cy7 (BD Pharmingen, Erembodegem, Belgium), KDR-APC
160 (R&D Systems, Minnesota, USA), CD45-APC-H7 (BD Pharmingen). Negative controls included
161 fluorescence-minus-one samples and unstained samples. The nucleic acid dye SYTO 13 (Life
162 Technologies, Gent, Belgium) allowed exclusion of non-nucleated cells and cellular debris. At
163 least 1 million total events were recorded on a FACSCanto II flow cytometer (Becton
164 Dickinson, New Jersey, USA). Numbers of EPC were analyzed using FACSDiva software
165 (Becton Dickinson, version 6.1.2) and expressed as cells per million CD45+ mononuclear cells
166 with low forward (FSC) and side scatter (SSC). Briefly, after exclusion of cellular aggregates
167 (FSC area versus height plot) and debris (SYTO 13 negative), a primary gate was set on the
168 mononuclear cells. Next, a second gate was set on a CD45 versus SSC dot plot to contain all
169 CD45^{dim} events, as previously described.(23) CD34+ and KDR+ events were analyzed in this
170 population.

171

172 Quantification of angiogenic T-cells

173 Angiogenic T-cells were defined as CD31+CD3+CD184+ cells.(14) After red cell lysis and Fc
174 receptor blocking at day 2 or 3, fixated whole blood was stained with CD31-FITC, CD3-PerCP,
175 and CD184-APC antibodies (all from BD Biosciences). Unstained samples and fluorescence-
176 minus-one samples for CD31 and CD184 were used as controls. At least 500.000 total events
177 were analyzed using FACSDiva software and expressed as cells per million mononuclear cells.
178 Doublets and aggregates were excluded by selecting singlet cells on a FSC area versus FSC
179 height plot.

180

181 Quantification of EMP

182 EMP were defined as CD31+CD42b- particles smaller than 1 μ m (Fluoresbrite YG 1 μ m
183 calibration size beads, Polysciences, Eppelheim, Germany). EMP enumeration was
184 performed only on samples collected at Center 1 (n= 90). For this purpose, platelet poor
185 plasma (PPP) was produced immediately after blood sampling by double centrifugation at
186 1550x g. Antibodies used were CD31-PE and CD42b-FITC (both from BD Biosciences).
187 Samples were analyzed as we previously described, enabling the evaluation of circulating
188 EMP per μ l PPP. (28)

189

190 Biochemical assays

191 Complete blood count was measured on Advia Haematology Analyzer (ADVIA 2120, Siemens
192 Healthcare Diagnostics). Levels of creatinine and high sensitivity C-reactive protein (hs-CRP)
193 were measured using routine laboratory techniques (Dimension Vista 1500 System,
194 Siemens). Estimated glomerular filtration (eGFR) was calculated using the MDRD formula.

195

196 ***Statistical analysis***

197 Continuous data are expressed as mean $\pm$ standard deviation (SD). Skewed distributed data
198 (one-sample Kolmogorov Smirnov test) are presented as median (range). Baseline
199 comparisons were performed using independent sample T test or χ^2 test where appropriate.
200 Differences over time between groups (=interaction) were analyzed by univariable two-way
201 repeated measures analysis of variance (ANCOVA) with age and pathology as covariates.
202 Whereas no centre-effect was found for peak VO₂, FMD values were significantly higher in
203 center 2. Therefore, ANCOVA for FMD included age, pathology and center as covariates.
204 Percentual changes of FMD were skewed and therefore expressed as median (range).
205 Pearson or Spearman correlation coefficients were used for correlations. A stepwise
206 multiple linear regression analysis was used to assess independent determinants of peak
207 VO₂ changes with adjustment for significant determinants on correlation analysis. All tests

208 were two-sided, and a p-value of 0.05 was considered statistically significant. All analyses
209 were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

210

211 **RESULTS**

212

213 ***Patient characteristics***

214 Demographic and clinical characteristics of the patients are shown in Table 1. Age and
215 pathology differed significantly, with younger age, more post-AMI and less post-PCI patients
216 in the AIT group, while other baseline values and pharmacological treatment were similar
217 between AIT and ACT.

218

219 ***Baseline levels of endothelial integrity markers***

220 Numbers of circulating EPC, angiogenic T-cells and EMP were not associated with the
221 presence of cardiovascular risk factors like hypertension, diabetes and smoking (all $p>0.05$).
222 In addition, numbers did not correlate with age, hs-CRP or eGFR (all $p>0.05$). There were no
223 differences in EPC, angiogenic T-cells and EMP numbers between post-AMI, post-PCI or post-
224 CABG patients (all $p>0.05$).

225

226 ***Changes in clinical parameters post-training***

227 As previously reported, mean training intensity was 88% of peak HR in the AIT group and
228 80% of peak HR in the ACT group.(5) Peak VO_2 was significantly higher in both groups after
229 12 weeks (AIT $22.7\pm17.6\%$ versus ACT $20.3\pm15.3\%$; $p\text{-time}<0.001$, Table 2). In addition, FMD
230 improved significantly in both groups (AIT median 34.1% (range -69.8 to 646%) and ACT
231 median 7.14% (range -66.7% to 503%); $p\text{-time}<0.001$). The improvement in peak VO_2 was
232 correlated to improvement in FMD (pearson $r = 0.17$, $p = 0.035$). Improvements in both
233 outcomes were comparable for both training interventions.

234

235 ***Changes in numbers of EPC, angiogenic T-cells and EMP post-training***

236 After 12 weeks of AIT or ACT, numbers of EPC, angiogenic T-cells and EMP in peripheral
237 blood were comparable to baseline levels (all $p > 0.05$, Table 2). This was observed for both
238 AIT and ACT groups. Results were comparable between the three different etiologies: we
239 observed no significant change in the number of EPC, angiogenic T-cells or EMP, neither in
240 the post-AMI group, nor in the post-PCI or post-CABG group (all $p > 0.05$). Various clinical (age,
241 BMI and systolic blood pressure) and biochemical variables (hs-CRP, eGFR, LDL cholesterol
242 and leukocytes) were evaluated for their possible interference effects on EPC, angiogenic T-
243 cells and EMP. None of these variables were related to changes in markers of endothelial
244 integrity (all $p > 0.05$).

245

246 ***Cellular markers as predictors of the training-induced response***

247 At baseline, no correlations were observed between cellular markers and endothelial
248 function or aerobic capacity. However, baseline numbers of EMP were related to the
249 magnitude of the change in peak VO_2 (spearman $\rho = -0.245$, $p = 0.027$) and the change in
250 FMD (spearman $\rho = -0.374$, $p = 0.001$) following exercise training. For this analysis, the
251 total cohort was pooled (AIT and ACT) since an interaction term was absent and EMP
252 numbers were logarithmically transformed. These relations were maintained in multivariate
253 regression analysis of logarithmically transformed EMP numbers and after correction for
254 baseline peak VO_2 and age, variables that were related to the change in peak VO_2 in
255 univariate analysis (beta -0.263 , $p = 0.01$). Baseline EPC or angiogenic T-cells did not
256 correlate with changes in FMD or peak VO_2 .

257

258

259

260 **DISCUSSION**

261

262 To our knowledge, the current substudy of the SAINTEX-CAD trial is the largest randomized,
263 multicenter study evaluating the effect of exercise training on cellular blood markers of
264 endothelial integrity in stable CAD patients. Despite a significant improvement in peripheral
265 endothelial function, we found no meaningful changes in the numbers of circulating EPC,
266 angiogenic T-cells and EMP after 12 weeks of AIT or ACT. EMP counts at baseline, however,
267 were related to the improvements in peak VO_2 and FMD at completion of the training
268 program.

269

270 **Blood related markers of endothelial damage and repair**

271 Endothelial dysfunction precedes overt atherosclerosis by many years and is an independent
272 prognostic marker of cardiovascular events.(22) Disruption of endothelial homeostasis
273 results from imbalances in the production of nitric oxide and reactive oxygen species, local
274 and systemic low-grade inflammation and loss of endothelial cells by apoptosis.(4) The injury
275 of the vessel wall leads to the recruitment of circulating EPC and angiogenic T-cells to the
276 site of endothelial disruption. It is well known from human and animal studies that EPC and
277 angiogenic T-cells, which stimulate EPC function, actively participate in the repair of
278 damaged endothelium.(29, 30) Moreover, endothelial repair seems to improve with their
279 increased numbers in the circulation, reflecting a higher regenerative capacity.

280 High levels of EMP affect the endothelial cell layer lining in a negative manner, in contrast to
281 EPC and angiogenic T-cells. EMP are shed from the plasma membrane of endothelial cells
282 upon their activation, apoptosis or injury.(12) These small particles may contribute to
283 worsening of endothelium injury by impairing the endothelium-dependent vasodilation and
284 modulating inflammation via leukocyte activation and trans-endothelial migration.(9)

285

286 **Impact of exercise training on endothelial function and endothelial repair**

287 This is the first study to examine the influence of exercise training on circulating levels of
288 EMP and angiogenic T-cells in patients with CAD. Data regarding the impact of physical
289 training on the number of EPC, however, are conflicting. One recent study reported an
290 increase in CD34+KDR+CD45dim EPC at the completion of a 4 week aerobic exercise training
291 program in 61% of stable patients with a previous AMI (n=112).(3) In that study, patients in
292 the lowest tertile of baseline hs-CRP were most likely of obtaining an increase in EPC.
293 Variations in peak VO₂ were correlated with variations in EPC, and patients without an
294 increase in peak VO₂ (n = 26) demonstrated a lower improvement in EPC number as
295 compared to patients with an increase in peak VO₂. Likewise, Ikeda et al. described that a 3
296 month walking program of > 4 hours walking per week, led to a gain in aerobic capacity and
297 CD34+CD133+ EPC number in patients with recent AMI (n=23).(15) Moreover, Steiner et al.
298 provided evidence supporting a role for increased CD34+KDR+CD133+ EPC in the
299 augmentation of endothelial function during exercise in CAD patients (n=20).(24) Three
300 months of aerobic exercise training resulted into a higher EPC level, which was positively
301 correlated with the change in FMD. Paul et al. also reported a rise in CD133+KDR+ EPC in 35
302 out of 46 CAD patients after three months of aerobic exercise training.(21) Brachial artery
303 FMD, however, was not improved and did not correlate with the number of EPC. In addition,
304 the reduction in plasma hs-CRP was modest and did not reach statistical significance at
305 program completion. Finally, Luk et al. (n = 32) and Hansen et al. (n = 47) did not find a
306 significant increase in CD34+KDR+ EPC in patients with CAD after 8 and 6 weeks of aerobic
307 exercise respectively.(11, 17) Although we must acknowledge the considerable variation in
308 EPC phenotypes, our results are more consistent with these latter studies, indicating that
309 EPC, angiogenic T-cells and EMP are not critically involved in mediating the training-induced
310 improvement of endothelial function in CAD patients.

311

312 **Responders to exercise training**

313 To date, it is widely recognized that the individual response to exercise training in terms of
314 aerobic capacity is highly variable among patients. Aerobic capacity is one of the strongest
315 prognosticators in cardiovascular disease, but 20% of patients have a low or absent response
316 in peak VO₂ to training.(1) The mechanisms driving this variability are not well understood
317 nor do we have good predictors of the response to exercise therapy. Heritability accounts
318 for 45–50% of the anticipated effect of exercise training.(2) In the present study, EMP count
319 at baseline was an important within-patient predictor of the change in peak VO₂ and
320 peripheral endothelial function at completion of a 3-month training program. Although
321 additional studies are needed to confirm its predictive value, EMP flow cytometry may offer
322 guidance to clinicians and physiotherapists in order to tailor exercise protocols to the need
323 of individual patients and thereby maximize the beneficial effects.

324

325 **Limitations**

326 The definition of EPC is still a matter of debate. In our study, EPC enumeration was
327 performed according to the recommendations of Van Craenenbroeck et al.(27) The
328 phenotypic profile of EMP may change according to the type of vascular injury (activation or
329 apoptosis). It is therefore unlikely that our set of markers efficiently labeled the entire EMP
330 population. A final limitation is that we could not include a functional analysis of circulating
331 angiogenic cells in this study design. Circulating angiogenic cells contribute to endothelial
332 repair in a paracrine fashion. In previous work, we found that exercise induces favorable
333 effects on the functional capacity of these cells in chronic heart failure patients.(26)

334

335 **Conclusions**

336 Our results demonstrate that the improvement of endothelial function in response to
337 exercise training in stable CAD patients is not mediated by an increased release of EPC and

338 angiogenic T-cells nor to a diminished shedding of EMP into the peripheral circulation. EMP
339 counts at baseline, however, may be predictive of the extent of increase in aerobic capacity
340 and endothelial function at completion of the training program.

341

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344 former leader of our research group, whose early death struck us deeply.

345

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474 **FIGURE LEGENDS**

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476 **Figure 1. Schematic illustration of both training programs.**

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485 **TABLES**

486

487 **Table 1. Demographic and clinical characteristics at baseline**

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	AIT n=100	ACT n=100	P-value
Age (yrs)	57.0 ± 8.8	59.9 ± 9.2	0.023
Gender (M/F)	91/9	89/11	NS
LVEF (%)	57.1 ± 8.5	56.8 ± 7.7	NS
Cardiovascular risk			
Body mass index (kg/m²)	28.0 ± 4.4	28.5 ± 4.3	NS
Diabetes (%)	20	18	NS
Hypertension (%)	58	46	NS
Smoking (%)	73	74	NS
Reason for referral			
CABG (%)	26	34	NS
Elective PCI (%)	7	18	0.019
AMI (%)	67	48	0.007
Laboratory measurements			
Hemoglobin (g/dl)	14.3 ± 1.3	14.2 ± 1.4	NS
Leukocytes (cells/μl)	6698 ± 1575	1042 ± 2094	NS
eGFR (ml/min/1.73 m²)	78.9 ± 12.9	77.5 ± 12.6	NS
hs-CRP (mg/l)	4.71 ± 9.4	3.45 ± 7.2	NS
Exercise Capacity			
Peak VO₂ (ml/kg/min)	23.3 ± 5.8	22.2 ± 5.6	NS
% VO₂predicted	82.8 ± 22.6	83.3 ± 22.7	NS
Maximal workload (Watts)	152 ± 39	144 ± 41	NS
Medication			
Statin (%)	97	99	NS
Aspirin (%)	93	95	NS
Beta-blocker (%)	84	83	NS
ACE-inhibitor/ARB (%)	77	72	NS

489 Values are mean (±SD) or percentage (%). NS: not significant.

490 LVEF= left ventricular ejection fraction; CABG= coronary artery bypass graft; PCI=
491 percutaneous coronary intervention; AMI= acute myocardial infarction; eGFR= estimated
492 glomerular filtration rate; hs-CRP= high sensitivity C-reactive protein; VO₂= oxygen uptake;
493 ACE= angiotensin converting enzyme; ARB= angiotensin II receptor blocker
494
495

496 **Table 2. Endothelial function and endothelial integrity markers following AIT or ACT**

	AIT		ACT		F value time	F value Interaction
	0 weeks	12 weeks	0 weeks	12 weeks		
Peak VO₂ (ml/kg/min)	23.5 ± 5.7	28.6 ± 6.9	22.4 ± 5.6	26.8 ± 6.7	28.18 *	0.16 (NS)
FMD (%)	5.26 ± 3.02	6.47 ± 2.79	5.61 ± 2.36	6.68 ± 3.04	7.28 *	0.06 (NS)
EPC (/10⁶ MNC)	8.2 (0-51)	7.4 (0-53)	9.5 (0-37)	10.6 (0-106)	0.13 (NS)	1.8 (NS)
Angiogenic T-cells (/10 ⁶ MNC)	1901 (186- 20494)	1950 (117- 26247)	2744 (132- 24000)	4765 (164- 31392)	0.95 (NS)	1.23 (NS)
EMP (/μl)	129 (47-756)	192 (47-755)	227 (80-715)	260 (60-922)	0.0 (NS)	0.8 (NS)

497 Values are mean ($\pm$ SD) or median (range) * p< 0.001, NS: not significant. EPC, angiogenic T-
 498 cells and EMP data were log transformed before analysis. ANCOVA with age and pathology
 499 as covariates was performed to test time and interaction effects.

500 AIT= aerobic interval training; ACT= aerobic continuous training; VO₂= oxygen uptake; FMD=
 501 flow mediated dilation; EPC= endothelial progenitor cells; EMP= endothelial microparticles;
 502 MNC= mononuclear cells.

503

AIT

ACT

AIT= Aerobic interval training; ACT= Aerobic continuous training; HR= heart rate