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BRIEF COMMUNICATION

Acute Exercise-Induced Circulating Haematopoietic Stem and Progenitor Cells in Cardiac Patients — A Case Series

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Background Exercise-induced circulating haematopoietic stem and progenitor cell (HPC) number has been discussed in the context of regeneration in heart disease patients.

Objective The aim of this pilot study was to compare the effect of different exercise protocols usually applied in cardiac rehabilitation on the number of acute, exercise-induced HPCs, related to potential mediators, e.g. biomarkers of sympathetic and oxidative stress, and inflammation.

Methods This is a case series comprising seven patients suffering from coronary heart disease (CHD) undertaken at the Center for Ambulant Cardiac Rehabilitation. Patients (n = 6) performed two exercise modes (constant-load, CLE; high-intensity interval, HIIE) in randomised order. Venous blood was drawn before and immediately after each test to assess CD34+/CD45+ HPC number by flow cytometry and biomarkers in blood plasma. The primary outcome was the change in HPC number, the secondary outcomes were changes in sympathetic/oxidative stress and markers of inflammation.

Results Both exercise modes resulted in a non-significant increase in HPC number after exercise, even when the results of both tests were combined. Overall, free norepinephrine increased significantly and was positively related to exercise-induced HPC number ($r = 0.70$, $p < 0.05$). Markers of sympathetic activation (fNE), oxidative stress (myeloperoxidase) and inflammation (interleukin-6) significantly increased after CLE and HIIE with no difference between tests.

Conclusions Interestingly, acute CLE and HIIE did not stimulate significant HPC mobilisation in CHD, although both exercise modes elevated circulating concentrations of sympathetic activation. Haematopoietic stem and progenitor cell mobilisation could be blunted due to disease-related bone-marrow exhaustion.

Keywords HPC mobilisation • Cardiac rehabilitation • β-adrenoreceptor antagonist

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Introduction

The potential therapeutic effect of circulating haematopoietic stem and progenitor cells (HPCs) on the ischaemic heart after acute coronary syndromes is believed to act via the support in turnover of vascular endothelium and myocardial repair [1]. Exercise mobilises HPCs to the peripheral blood–among others–via a catecholaminergic mechanism [2], where free norepinephrine (fNE) and epinephrine (fEPI) are the active agents. Data in healthy individuals [3] suggest a doubling of HPC number after acute, exhaustive exercise. Conversely, data in heart disease patients are rare, although the contribution of exercise to cardiac regeneration based on mobilised precursors is promising [4,5]. Unfortunately, the importance of the sympathetic system for mobilisation, is often neglected [5], especially since HPC mobilisation seems to be dependent on a β_2-adrenergic mechanism [6]. Both constant-load continuous (CLE) and high-intensity interval (HIIE) exercise training were effective in mobilising HPCs [7] and have been recommended for cardiac rehabilitation [8]. Although the exact exercise-induced HPC response in patients undergoing cardiac rehabilitation is still a question of debate, one can possibly hypothesise similar trigger effects from CLE and HIIE [9].

In addition to sympathetic activation (fNE, fEPI, cortisol (Co) [2]), oxidative stress (malondialdehyde (MDA), myeloperoxidase (MPO) [10]), and inflammation (interleukin-6; IL-6 [2]) play a role in HPC mobilisation in the healthy and should also be considered in heart disease.

The aim of this case series was to determine the effects of CLE- and HIIE-induced stress/inflammatory responses on HPC number. We hypothesised that CLE and HIIE would show a comparable increase of HPCs related to plasma catecholamine concentrations.

Case Series

Subjects

Patients in cardiac rehabilitation (NYHA I, male = 6, female = 1, age: 64.6 ± 7.1yrs) were recruited [9]. They were assigned such that each exercise mode was performed by at least six subjects. Due to logistic reasons only five patients performed both exercise tests. All of the subjects had coronary heart disease. Preceeding coronary incidences had occured >8 weeks before study onset and left ventricular ejection fraction was ≥45%. Medication of patients were β_1-adrenoreceptor antagonists (85.7%), statins (85.7%), ACE inhibitors (85.7%), antiplatelet agents (100%), and proton pump inhibitors (85.7%) which were kept constant during the study period. All participants gave their informed consent. The study design was approved by the ethics commitee of the local Medical University (EK-decision-number 23-397ex10/11).

Exercise Testing

Constant-load continuous and HIIE were matched for mean power output (P_{mean}) and total exercise duration [9]. All tests were performed on a cycle ergometer ≥2 days apart. An incremental exercise was performed to obtain the first (LTP_1) and the second (LTP_2) lactate turn points to prescribe the target workload [9] for CLE and HIIE, which were carried out in a randomly assigned order. CLE was performed at P_{mean} (=20% below LTP_2) for 28 minutes. Power output during high-intensity intervals was set at maximum power output of the incremental exercise test (P_{peak}) and duration was 20 s (t_{peak}). Recovery workload after each interval (P_{rec}) was low-intensity at a power

Table 1 Exercise variables overview.

	Exercise Tests		
	IE	*CLE*	*HIIE*
$La_{max/peak}$ (mmol/l)	6.56 ± 2.94	3.45 ± 1.34	3.18 ± 1.64
La_{mean} (mmol/l)	2.47 ± 0·87	2.95 ± 1.08	2.72 ± 1.40
$HR_{max/peak}$ (b/min)	138.4 ± 29.4	101.0 ± 5.5	109.8 ± 20.4
HR_{mean} (b/min)	99.9 ± 16·09	94.0 ± 7.8	99.9 ± 18.3
$P_{max/peak}$ (W)	146.0 ± 52.5	68.5 ± 6.4	133.2 ± 31.3
P_{mean} (W)	78.3 ± 26.2	68.5 ± 6.4	75.8 ± 17.4
$VO2_{max/peak}$ (l/min)	1.82 ± 0.69	1.43 ± 0.13	1.53 ± 0.20
$VO2_{mean}$ (l/min)	1.16 ± 0·26	1.30 ± 0.13	1.37 ± 0.19
P_{LTP1} (W)	53.0 ± 12.4	–	–
P_{LTP2} (W)	104.0 ± 35.0	–	–
EE (kcal)	87.7 ± 52.6	187.2 ± 18.6	187.1 ± 23.2

Values are reported as mean ± standard deviation, n = 6 (exceptions: HR_{max} of IE n = 5; HR_{peak} and HR_{mean} of CLE and HIIE n = 4).

Comment: P_{LTP1} and P_{LTP2} are only deductible for IE.

Abbreviations: IE, incremental exercise; CLE, constant-load exercise; HIIE, high-intensity interval exercise; La, blood lactate concentration; HR, heart rate; P, power output; VO2, oxygen uptake; P_{LTP1}, lactate turn point 1; P_{LTP2}, lactate turn-point 2; EE, energy expenditure.

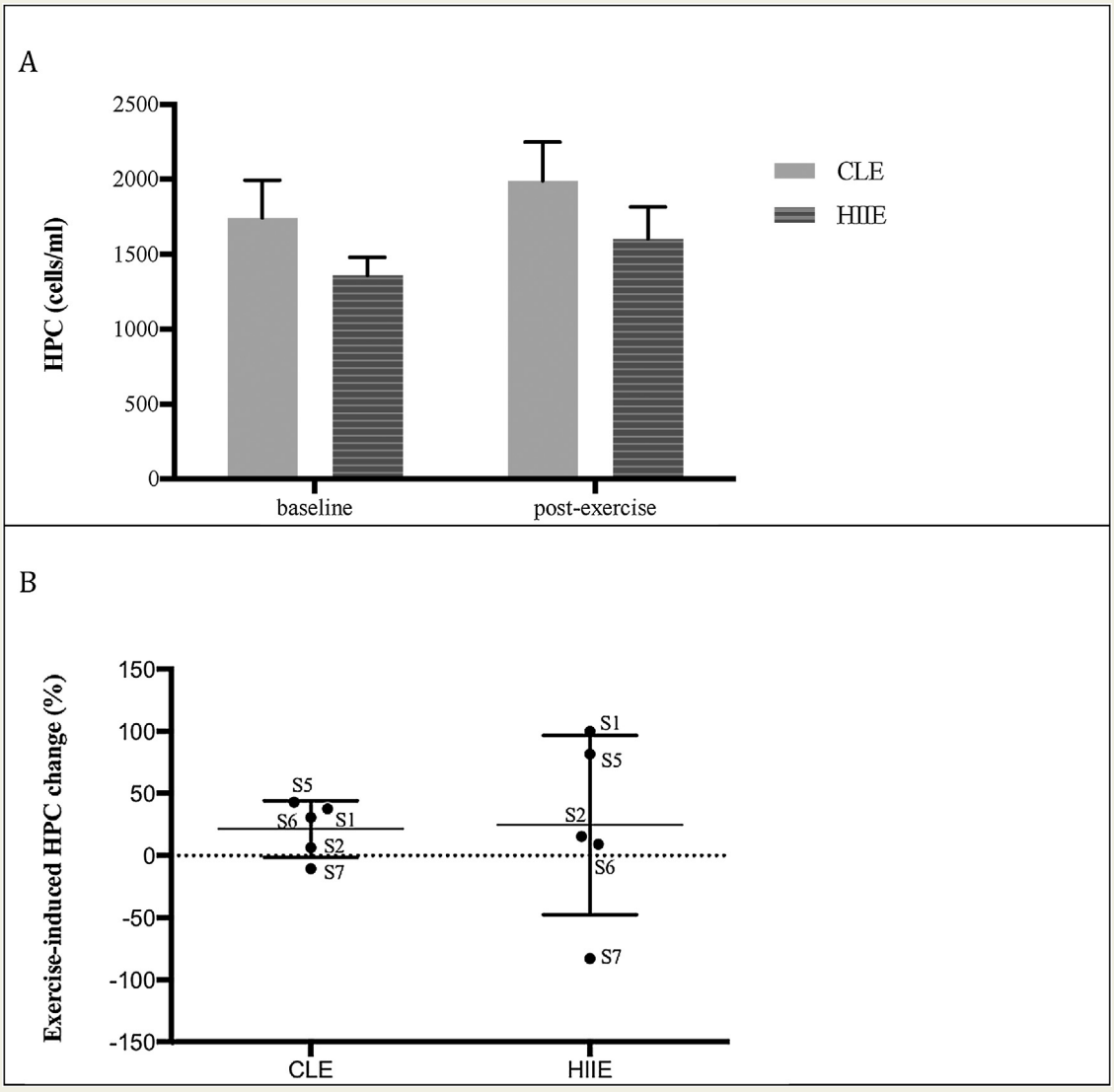

Figure 1 Exercise-Induced HPC number.
A. Absolute circulating haematopoietic stem and progenitor cells (CD34+/CD45+) before (baseline) and after (post-exercise) CLE and HIIE; n = 6
B. Exercise-induced change in circulating haematopoietic stem and progenitor cells (CD34+/CD45+) for both CLE and HIIE; n = 5
Abbreviations: CLE, constant-load continuous exercise; HIIE, high-intensity interval exercise.

output of 10% below LTP_1. The following equation was applied to calculate the necessary variables for HIIE: $P_{mean} = \frac{P_{peak} \cdot t_{peak} + P_{rec} \cdot t_{rec}}{t_{peak} + t_{rec}}$.

This implies that t_{rec} and the number of intervals differed for each subject. The duration of recovery ranged from 30 to 50 seconds and the number of intervals from 24 to 34. Each test was followed by a 5-minute cool-down at 0W. Before the test and immediately after the cool-down phase, 18 ml of venous blood were withdrawn from the antecubital vein.

Blood Processing

Blood was collected in lithium-heparinised tubes (Greiner Bio-One, Kremsmünster, Austria) for determination of IL-6, and cortisol levels and in ammonium-heparinised tubes containing 1.25 mg gluthatione for catecholamine levels. To determine oxidative stress markers, EDTA samples (Greiner Bio-One, Kremsmünster, Austria) were used. Samples were centrifuged (~3000 rpm, 10 min), plasma was removed, immediately frozen and stored at −80 °C until analysis. EDTA whole blood was used to analyse CD34+/CD45+ (BD Biosciences, Vienna, Austria) HPCs by flow cytometry (FACSCalibur, BD Biosciences, Vienna, Austria) and blood cell counts by a haematocytometer (Sysmex Corporation, Kobe, Japan) [10]. Plasma parameters were analysed as previously described [2,10].

Table 2 Plasma parameters before and after CLE and HIIE.

	Constant-load Exercise			High-intensity Interval Exercise		
	Before	*After*	*Delta %*	*Before*	*After*	*Delta %*
fNE, pg·ml^{-1}	1067.3 ± 202.3	1894.0 ± 442.3*	77.5	992.5 ± 282.7	2242.1 ± 699.45*	125.9
bNE, pg·ml^{-1}	3140.7 ± 430.9	3569.8 ± 601.01	13.7	3163.1 ± 617.0	4546.4 ± 725.1	43.7
fEPI, pg·ml^{-1}	64.8 ± 5.7	112.3 ± 17.0	73.3	119.9 ± 42.2	171.3 ± 42.1*	42.9
bEPI, pg·ml^{-1}	265.9 ± 108.7	255.3 ± 93.3	-4.0	142.2 ± 3.4	177.4 ± 43.1	24.8
Co, ng·ml^{-1}	137.05 ± 5.59	148.02 ± 15.02	8.0	116.62 ± 15.87	138.70 ± 27.27	18.9
MDA, µmol·l^{-1}	0.53 ± 0.11	0.50 ± 0.06	−5.7	0.55 ± 0.08	0.47 ± 0.05	−14.5
MPO, µg·l^{-1}	17.8 ± 4.8	24.3 ± 6.9*	36.5	18.2 ± 5.0	26.7 ± 7.1*	46.7
IL-6, pg·ml^{-1}	4.88 ± 0.97	6.01 ± 0.98*	23.2	4.67 ± 0.78	6.00 ± 0.97*	28.5

Values are mean ± standard deviation, n = 6 (exceptions: MDA baseline n = 5 and gEPI baseline n = 3 for CLE). Delta %, percentage change from baseline to post.
Significant differences between time points are indicated as follows: *p < 0.05 in comparison to baseline values.
Abbreviations: f/b NE/EPI, free/bound norepinephrine/epinephrine; Co, cortisol; MDA, malondialdehyde; MPO, myeloperoxidase; IL-6, interleukin-6.

Statistics

Data were reported as mean ± standard deviation (SD) unless otherwise indicated. Sample size was based on a-priori results of HIIE in healthy subjects having the hypothesis that HPCs would significantly increase after a HIIT protocol [7] with an effect size of 1.28 and an actual power of 0.83, where n = 6 was considered appropriate for a case series. Furthermore, for the comparison of exercise-induced HPC number between CLE and HIIE, a-priori power calculations [7] showed that n = 5 would have sufficient statistical power (1-beta = 0.80) using an effect size of 1.77. Related-samples Wilcoxon signed-rank tests were performed for comparisons. Pearson's correlation analysis was used to test an overall relationship between parameters. IBM SPSS Statistics21 and G*Power3.1.7 were used for analysis. A p-value <0.05 was considered significant.

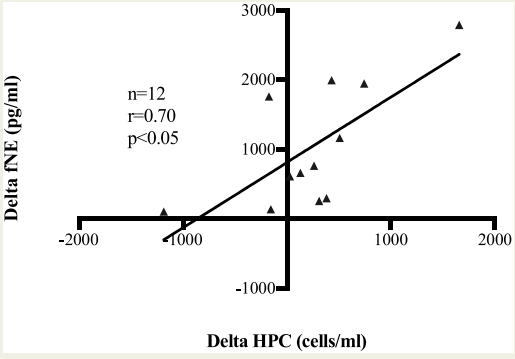

Figure 2 Relationship of exercise-induced HPC and free norepinephrine changes.
Pearson's correlation analysis between mobilised haematopoietic stem and progenitor cell (HPC) number and exercise-induced free norepinephrine (fNE) concentrations (n = 12). There was a strong relationship between mobilised HPC number and exercise-induced fNE (r = 0.70, p < 0.05), which implies a triggering effect of fNE on haematopoietic precursor cells in patients recovering from coronary incidences taking β_1-adrenoreceptor antagonist medication.

Results

An overview of derived exercise variables can be found in Table 1. There was no significant difference in peak or mean blood lactate concentration, heart rate, oxygen uptake or energy expenditure between CLE and HIIE. Neither CLE, nor HIIE alone increased the absolute CD34+/CD45+ cell number significantly (p > 0.05, Figure 1A). Exercise-induced HPC number was also not significantly elevated when data of both tests were combined. Constant-load continuous exercise significantly increased fNE, MPO and IL-6 (all p < 0.05) and HIIE significantly increased fNE, fEPI, MPO and IL-6 (all p < 0.05, Table 2). Baseline values as well as exercise-induced changes in HPC number (Figure 1B), free and bound catecholamines, MDA, MPO and IL-6 did not significantly differ between CLE and HIIE (p > 0.05).

Combining data of both tests, we found a significant relationship between the exercise-induced increase of HPCs (Delta HPC) and the increase in fNE (Delta fNE) (r = 0.70, p < 0.05, Figure 2).

Discussion

Although it is still controversial as to whether the increase in peripheral HPCs will result in new myocardial cells, especially in the injured heart [11], there is clear evidence that circulating bone marrow-derived haematopoietic cells participate in cardiomyocyte regeneration after an acute coronary event [12] and their regenerative capacity is not negatively affected [13]. This is why it can be hypothesised that physical exercise supports heart regeneration and patient recovery–among other

factors–also by progenitor mobilisation [5]. The present case series in cardiac patients revealed a non-significant 0.1 to 0.2-fold increase of HPC after CLE, and HIIE together with a significant 0.3 (CLE) to 0.6 (HIIE) -fold increase in total NE, while fNE/bNE increased 0.5-fold after both tests. This contrasts investigations in young [2,6,7] and elderly [14] healthy subjects. Krüger et al. [7] found on average a significant HPC rise after both HIIE and CLE (0.4- and 0.5-fold, respectively), while total NE significantly increased 0.6 (CLE) to 0.9 (HIIE) -fold. Agha et al. [6] stated that β_1-receptor antagonist treatment (as often used in cardiac rehabilitation) would not blunt exercise-induced CD34+ cell mobilisation. This contradicts decreased HPC mobilisation by a medication-induced blunted sympathetic system and possibly implies an altered bone-marrow microenvironment (e.g. bone-marrow exhaustion) [15], supported by the significant relationship between exercise-induced HPC number and fNE changes in our study.

Conclusion

Matched constant-load continuous and HIIE did not significantly stimulate HPC mobilisation in heart disease patients, possibly due to disease-related bone-marrow exhaustion. In addition, CLE and HIIE had a comparable effect on sympathetic and oxidative stress and inflammatory biomarkers. Results of this case series emphasise the necessity of in-depth knowledge of exercise-induced HPC mobilisation and possible influencing factors such as attenuated mobilisation due to disease-related bone-marrow alterations, prescribed medication or exercise prescription in patients undergoing cardiac rehabilitation.

Study Limitations

Although results of this case series were preliminary due to the small patient number, a-priori sample size estimation for future studies revealed no change in outcome up to the inclusion of 169 patients (calculated from pooled CLE and HIIE results using a one-tailed paired t-test, an effect size of 0.19 and an expected power of >0.8).

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Declaration of Interest

None.

References

[1] Wojakowski W, Landmesser U, Bachowski R, Jadczyk T, Tendera M. Mobilization of stem and progenitor cells in cardiovascular diseases. Leukemia 2012;26:23–33.
[2] Kropfl JM, Stelzer I, Mangge H, Pekovits K, Fuchs R, Allard N, et al. Exercise-induced norepinephrine decreases circulating hematopoietic stem and progenitor cell colony-forming capacity. PLoS One 2014;9: e106120.
[3] De Lisio M, Parise G. Exercise and hematopoietic stem and progenitor cells: protection, quantity, and function. Exerc Sport Sci Rev 2013;41:116–22.
[4] Figueiredo PA, Appell Coriolano HJ, Duarte JA. Cardiac regeneration and cellular therapy: is there a benefit of exercise. Int J Sports Med 2014;35:181–90.
[5] Guo Y, Ledesma RA, Peng R, Liu Q, Xu D. The Beneficial Effects of Cardiac Rehabilitation on the Function and Levels of Endothelial Progenitor Cells. Heart Lung & Circulation 2017;26:10–7.
[6] Agha NH, Baker FL, Kunz HE, Graff R, Azadan R, Dolan C, et al. Vigorous exercise mobilizes CD34+ hematopoietic stem cells to peripheral blood via the beta2-adrenergic receptor. Brain Behav Immun 2018;68:66–75.
[7] Kruger K, Alack K, Ringseis R, Mink L, Pfeifer E, Schinle M, et al. Apoptosis of T Cell Subsets after Acute High-Intensity Interval Exercise. Med Sci Sports Exerc 2016;48:2021–9.
[8] Gayda M, Ribeiro PA, Juneau M, Nigam A. Comparison of Different Forms of Exercise Training in Patients With Cardiac Disease: Where Does High-Intensity Interval Training Fit. Can J Cardiol 2016;32:485–94.
[9] Tschakert G, Kroepfl JM, Mueller A, Harpf H, Harpf L, Traninger H, et al. Acute Physiological Responses to Short- and Long-Stage High-Intensity Interval Exercise in Cardiac Rehabilitation: A Pilot Study. J Sports Sci Med 2016;15:80–91.
[10] Kroepfl JM, Pekovits K, Stelzer I, Fuchs R, Zelzer S, Hofmann P, et al. Exercise increases the frequency of circulating hematopoietic progenitor cells, but reduces hematopoietic colony-forming capacity. Stem Cells Dev 2012;21:2915–25.
[11] Ruiz-Villalba A, Simon AM, Pogontke C, Castillo MI, Abizanda G, Pelacho B, et al. Interacting resident epicardium-derived fibroblasts and recruited bone marrow cells form myocardial infarction scar. J Am Coll Cardiol 2015;65:2057–66.
[12] Wu JM, Hsueh YC, Ch'ang HJ, Luo CY, Wu LW, Nakauchi H, et al. Circulating cells contribute to cardiomyocyte regeneration after injury. Circ Res 2015;116:633–41.
[13] Kropfl JM, Spengler CM, Frobert A, Ajalbert G, Giraud MN. Myocardial infarction does not affect circulating haematopoietic stem and progenitor cell self-renewal ability in a rat model. Exp Physiol 2018;103:1–8.
[14] Thijssen DH, Vos JB, Verseyden C, van Zonneveld AJ, Smits P, Sweep FC, et al. Haematopoietic stem cells and endothelial progenitor cells in healthy men: effect of aging and training. Aging Cell 2006;5:495–503.
[15] Kissel CK, Lehmann R, Assmus B, Aicher A, Honold J, Fischer-Rasokat U, et al. Selective functional exhaustion of hematopoietic progenitor cells in the bone marrow of patients with postinfarction heart failure. J Am Coll Cardiol 2007;49:2341–9.