Abstract

Background: The aim of the present study was to determine whether regular exercise training (ET) is effective at promoting the mobilization of CPCs and improving their functional activity in patients with recently acquired myocardial infarction (STEMI). Regular physical training has been shown to improve myocardial perfusion and cardiovascular function. This may be related in part to a mobilization of bone marrow-derived circulating progenitor cells (CPCs) as well as an enhanced vascularisation.

Methods: 37 patients with STEMI were randomly assigned to an ET group or a non-ET group (controls). Two weeks after STEMI, three weeks after regular ET and three months after ET, BNP levels, exercise echocardiography and exercise spiroergometry were evaluated. The number of CD34+/CD45+ and CD133+/CD45+ CPCs was measured by flow cytometry analysis. The migration capacity of the CPCs was determined with a boyden chamber and the clonogenic capacity by CFU-assay.

Results: In the ET group the number and migration capacity of CPCs increased significantly after regular exercise training. The BNP level decreased significantly from 121 ± 94 to 75 ± 47 pg/ml (p<0.001) after the ET period, the left ventricular ejection fraction raised in parallel at peak exercise, and the cardiorespiratory condition improved as demonstrated by an increase of VO2max (from 1641 ± 522 to 1842 ± 724 ml/min, p<0.02). These three effects persist till three months after the ET period.

Conclusions: Regular physical activity appears to predispose the mobilization and enhanced functional activity of CPCs, a phenomenon which might lead to an improved cardiac function in patients with recently acquired acute myocardial infarction.

Key words: Exercise training, Cytokines, Progenitor Cells, Myocardial Infarction, Rehabilitation
been revealed following repeated exercise induced myocar
dial ischemia, as well as an enhanced expression of
angiogenic cytokines such as VEGF. This increases
the mobilisation of progenitor cells from the bone
marrow into the peripheral blood and probably leads
on the one hand to an intensified repair of vascular le-
sions in the coronary vasculature and on the other
hand to a neovascularisation within the heart muscle
[15].

The aim of our prospective study was to investigate
the effect of regular exercise training over three weeks
(rehabilitation program) on physical capacity, cardiac
performance and the mobilisation and function of
CPCs in patients with an acute myocardial infarc-
tion (STEMI). For this purpose we determined the quantity
and function of CPCs in peripheral blood isolated
from STEMI patients at three different time points: (t0) bevor intervention (2 weeks after acute myocardial
infarction) (t1) three weeks after intervention start
(five weeks after acute myocardial infarction) and (t2)
three months after rehabilitation (17 weeks after acute
myocardial infarction).

**PATIENTS AND METHODS**

**Study protocol:** Patients with a documented ST-eleva-
tion myocardial infarction (STEMI), onset of pain up
to 12 h, a left ventricular ejection fraction below
<60% and under the age of 75 years were enrolled in
this prospective study between February 2005 and Au-
gust 2006. Exclusion criteria included STEMI, signifi-
cant valvular heart disease, cardiogenic shock, renal
failure, anemia, orthopedics or other conditions that
prohibited participation in the exercise training. None
of the patients had a history of myocardial infarction.

All patients were treated immediately during the
acute phase of the infarction with aspirin, clopido-
grel and tirofiban. Subsequently coronary angio-
graphy was performed and the occluded infarct-relat-
ed coronary artery was recanalized. One week after
myocardial infarction all patients were randomly as-
signed to the exercise or non-exercise training group
(Fig. 1). For therapeutic reasons more patients were
randomized to the exercise training group (rehabilita-
tion) and so we chose a 2:1 randomization. At dis-
charge all patients received aspirin, clopidogrel, an-
giotensin-converting enzyme inhibitors or angio-
tensin-1 receptor antagonists, beta-blockers and
statins.

Follow-up visits were performed at 2 weeks (t0),
five weeks (t1) and 17 weeks (t2) and included clinical
status, laboratory examinations, ECG, exercise echo-
cardiography, spiroergometry, adverse events and
medications. The protocol for this study was approved
by the local Ethics Committee of the Heinrich-Heine
University in Düsseldorf.

**Study objectives:** The primary end points were changes
of global cardiac function using exercise echocardiog-
raphy from t0 (2 weeks after acute PCI) to t1 and t2
(five and 17 weeks) of follow-up. Global function was
determined by left ventricular ejection fraction (EF).
Secondary end points comprised changes of oxygen
consumption per unit time (VO2) using spiroergome-
ty from t0 (2 weeks after acute AMI) to five and 17
weeks of follow-up. Changes of mobilized stem cells
(CD34/CD45+, CD133/CD45 +), changes of migra-
tion capacity of the mononuclear cells and changes
of their clonogenic potential (CFU-assays) were ana-
yzed.

![Fig. 1. Enrollment and outcomes.](image-url)
Maximal exercise training test: A maximal exercise training (ET) test was performed on a calibrated, electronically braked bicycle ergometer in an upright position. It was performed once daily, on five days a week, and for three weeks in total (15 times maximal exercise training, Fig. 2).

According to Gysan et al. and Schönstedt et al., we chose an ambulatory shortterm endurance training, demonstrating good compliance by the patients and a beneficial outcome [16, 17]. ECG tracing and blood pressure readings were obtained every two minutes. Workload was increased progressively every 2 minutes at steps of 25 W, beginning at 25 W. Myocardial ischemia was confirmed by the presence of typical angina pectoris or a significant exercise-induced ST-segment depression (>0.1 mV descending or horizontal). Exercise was terminated when patients experienced progressive chest pain, physical exhaustion, or when a 3-mm horizontal ST-segment depression was reached.

Exercise echocardiography: Echocardiographic images were obtained in the parasternal long- and short axis, and apical 2- and 4-chamber views using commercially available equipment with a 2.5-MHz transducer rate of 30 frames/s. These images were recorded in quad-screen cine loop format before and within 90 seconds after treadmill exercise testing. Enddiastolic and endsystolic diameter of the left ventricle at rest and after exercise was visually interpreted by the consensus of at least two experienced physician echocardiographers unaware of the results of the left ventricular angiogram and the coronary angiography. The digital quadscreen cine loop format was reviewed for analysis of left ventricular function which was assessed by calculation of the enddiastolic and endsystolic volumes (Simpson formula).

Bicycle spiroergometry: After resting for 5 minutes, the patients had their arterial blood pressure measured at the right brachial artery using a sphygmomanometer. A 12-lead electrocardiogram was recorded, and resting heart rate (HR) was calculated from the R-R interval. Each subject underwent maximal exercise testing on a computer-driven bicycle ergometer using a ramp protocol starting at 50 W, with gradual increases of 25 W every 2 minutes. During the test, patients wore a tight-fitting face mask connected to an Oxycon Pro spirometer. Oxygen consumption per unit time (VO2), carbon dioxide production (VCO2), and minute ventilation were measured on a breath-by-breath basis. Subjects were exercised to their self-determined maximal capacity or until the physician stopped the test because of symptoms such as chest pain or dizziness, potentially dangerous arrhythmias or ST-segment deviations, or marked systolic hypotension or hypertension. A respiratory exchange ratio (VCO2/VO2) >1 was taken to indicate maximal effort. Maximum oxygen consumption was defined as the highest VO2 obtained at the end of the test and is expressed in ml/min and ml/min per kg.

Collection of venous blood samples: On the day before the exercise training program starts (t0), one day after the exercise training program ends (t1 – three weeks, 15 times exercise training tests) and three months after the exercise training program (t2) venous blood samples were taken in the morning from fasting patients. FACS analysis, isolations of circulating progenitor...
cells for migration and colony-forming-unit assay were performed in all samples.

From the control patients with no exercise training venous blood samples were also taken at matched time points (Fig. 2).

**Measurement of brain natriuretic peptide (BNP) in peripheral blood:** In order to determine BNP levels, peripheral blood was taken at the same three time points as described above. Whole blood samples were used from all patients and BNP was measured using Triage-Meter Plus (Biosite).

**Isolation and cultivation of circulating progenitor cells:** 20 ml peripheral venous blood was taken from each patient at the three time points, as described above. Blood was taken using a BD Vacutainer CPT system containing a citrate anticoagulant with Ficoll Hypaque density fluid and a polyester gel barrier. Blood samples were centrifugated for 20 min. at 3,000 rpm (rounds per minute) at room temperature. The mononuclear cells (MNCs) were resuspended by inverting the tube and the entire content above the gel was transferred into a separate tube. After adding phosphate buffered saline (PBS) the MNCs were centrifugated for 10 min. with 2,300 rpm. Afterwards ammonium chloride was added for 10 min. to accomplish the lysis of all red blood cells. After two washing steps with PBS mononuclear cells were resuspended in 1ml EBM2-medium (Cell systems). The cell number was determined in a Neubauer chamber.

**Flow cytometry analysis:** Two colour flow cytometry assays were performed using an EPICS XL flow cyto-

![Fig. 3. Representative flow cytometry gating strategy for CD45+/CD34+.](image-url)
Instrumentation:

The instrument was calibrated using beads (Beckman Coulter / Immunotech) according to the manufacturer’s instructions. For erythrocyte lysis, peripheral blood samples were diluted in 15 ml bicarbonate-buffered ammonium chloride solution (0.15M NH₄Cl, 0.01M NaHCO₃, 1.0mM EDTA) for 15 minutes at room temperature. The cells were centrifuged and resuspended in 500µl pH 7.2 phosphate-buffered saline (PBS) (lyse-no-wash, technique). In brief, 100µl of white-blood cell sample were incubated with 10µl of FITC-conjugated anti-CD45 (BD) and 10µl of phyocerythrin (PE)-conjugated anti-CD34 (BD) or anti-CD133 (BD) for 20 minutes at 4°C. All experiments included samples incubated with an isotype control PE-labelled IgG1 (Beckman Coulter) as a negative control for nonspecific binding. All samples were run in duplicate. Samples were stored on ice in the dark and analyzed within two hours. The ISHAGE sequential gating strategy exploits the fact that blast cells can be identified from their dim CD45 expression and low side-scatter (SSC). Figure 3 illustrates an example of our experiments using the gating strategy for CD34 analysis (Fig. 3a-f).

Migration assay:

Analysis of cell migration was performed by seeding 1x10⁶ MNCs per ml in the top compartment of a boyden chamber (Fig. 4). The bottom compartment contained either only EBM-2 medium (supplemented with 0.1% BSA) or 100ng/ml stromal cell derived factor-1 (SDF-1) or 200ng/ml VEGF (vascular endothelial growth factor) in EBM2-medium (supplemented with 0.1% BSA). Cells were allowed to migrate for 24h at 37°C in humidified atmosphere. The migrated cells were stained using a Diff-Quick Staining Kit (Dade Behringer) and then counted by two independent investigators.

Colony-forming unit assay:

1x10⁵ MNCs per ml were seeded in Methocult GF H4434 (Stemcell Technologies). Culture dishes were seeded with 1 ml cell suspension and then incubated at 37°C in 5% CO₂. Colony-forming-unit erythroid (CFU-E) and CFU-granulocyte/macrophage (CFU-GM) were evaluated 14 days after seeding, using an inverted microscope. The different clusters in the CFU assays were distinguished by their characteristic morphology and illumination by using an inverted microscope. No staining or immunostaining was performed. CFU-erythroid clusters showed small clusters and emitted a red to brownish colour, whereas CFU-granulocyte/macrophage clusters presented flat colonies consisting of translucent cells. CFU assays were quantified by counting the clusters by two independent investigators.

Statistics:

Variables are presented as means ± standard deviation (SD), depicted in the text and figures. Categorical variables were compared with use of the chi-square test or Fischer’s exact test. Statistical comparisons between initial and follow-up data were performed in a nonparametric paired fashion using the Wilcoxon signed-rank test. The nonparametric Mann-Whitney U test was used for intergroup comparisons. Statistical significance was assumed if p was < 0.05. All probability values are 2-tailed. Statistical analysis was performed using SPSS (version 12.0).

RESULTS

Patient characteristics:

The baseline characteristics were comparable in both groups (Table 1). All patients suffered from an occlusion (Thrombolysis in Myocardiac Infarction (TIMI) grade 0).
dial Infarction (TIMI) flow grade 0/1 of 1 coronary artery resulting in extensive myocardial infarctions. Percutaneous coronary intervention was successfully achieved in all patients resulting in TIMI flow grade 2/3. There was no significant difference between the exercise training group and the control group regarding cardiovascular risk factors, use of glycoprotein IIb/IIIa antagonists and current medication. 25 patients were randomly assigned to the exercise training group and 12 patients to the control group with no physical training.

Effects of exercise training on clinical parameters and BNP-levels in patients with STEMI: Patients in the exercise training group showed a significant improvement in their clinical condition after the exercise training test and three months later, as assessed by the NYHA classification (New York Heart Association). Furthermore, the BNP-level in peripheral blood decreased significantly after the ET-program from 121 ± 94 to 75 ± 47 pg/ml (p<0.001) and remained reduced three months thereafter at 54 ± 42 pg/ml (Table 2). There were no significant changes in the non-exercise control group. In the exercise echocardiography we found no difference in the left ventricular ejection fraction (EF) at rest in either group over the investigated time, but at peak exercise the left ventricular ejection fraction increased after the three-week-long exercise training program from 57 ± 14 to 60 ± 14%, and compared to the EF at rest (49 ± 12%), the peak EF (60 ± 14%) was significantly increased (p = 0.031). In the spiroergometry, VO2max increased significantly only in the ET-group from 1641 ± 522 to 1842 ± 724, p = 0.047 (baseline to after exercise training) and remained stable for the three months thereafter.

### Table 1. Characteristics of patients.

| Characteristics                                      | Exercise Training after AMI | Control- Patients with AMI | p    |
|-------------------------------------------------------|-----------------------------|-----------------------------|------|
| No. of patients                                       | 25                          | 12                          | n.s. |
| Gender m/w                                            | 21/4                        | 7/5                         | n.s. |
| Age (yrs)                                             | 60 ± 9                      | 63 ± 10                     | n.s. |
| Body-Mass Index (kg/m2)                               | 27.30 ± 2.43                | 27.4 ± 3.94                 | n.s. |
| NYHA classification                                    | 2.64 ± 0.97                 | 2.75 ± 0.62                 | n.s. |
| 1/II/III/IV                                           | 0/10/14/1                   | 0/4/7/1                     | n.s. |
| BNP level, pg/ml                                      | 121 ± 94                    | 127 ± 78                    | n.s. |
| Coronary angiography                                  |                             |                             |      |
| No. of coronary vessel disease                         | 2.16 ± 0.83                 | 2.5 ± 0.71                  | n.s. |
| 1 / 2 / 3 - coronary vessel disease                    | 7 / 8 / 10                  | 2 / 3 / 7                   |      |
| Infarct-related coronary vessel                        |                             |                             |      |
| LAD/LCX/RCA                                           | 10 / 7 / 8                  | 5 / 3 / 4                   |      |
| No. of patients with stent, n (%)                      | 24 (96)                     | 12 (100)                    |      |
| Risk factors, n (%)                                    |                             |                             |      |
| Smoking                                               | 10 (40)                     | 5 (42)                      | n.s. |
| Hypercholesterolemia                                  | 25 (100)                    | 12 (100)                    | n.s. |
| Diabetes mellitus                                     | 4 (16)                      | 2 (17)                      | n.s. |
| Hypertension                                          | 18 (72)                     | 9 (75)                      | n.s. |
| Positive family History                               | 8 (32)                      | 4 (33)                      | n.s. |
| Medication, n (%)                                     |                             |                             |      |
| Acetylsalicylic acid                                  | 25 (100)                    | 12 (100)                    | n.s. |
| Clopidoogel                                           | 24 (96)                     | 12 (100)                    | n.s. |
| Beta-blocker                                          | 25 (100)                    | 12 (100)                    | n.s. |
| ACE-inhibitor or AT-1 antagonist                       | 25 (100)                    | 11 (90)                     | n.s. |
| Statin                                                | 25 (100)                    | 12 (100)                    | n.s. |
| Laboratory parameters                                 |                             |                             |      |
| CPK [U/L]                                             | 1196 ± 1463                 | 1082 ± 1099                 | n.s. |
| CPK-MB (U/L)                                          | 189 ± 259                   | 136 ± 219                   | n.s. |
| Troponin I (ng/ml)                                    | 21.3 ± 15.3                 | 12.4 ± 9.2                  | n.s. |
| LDH (U/L)                                             | 549 ± 551                   | 319 ± 164                   | n.s. |
| CRP (mg/dl)                                           | 5.29 ± 7.53                 | 3.98 ± 5.41                 | n.s. |
The NYHA classification showed a significant improvement at five weeks and 17 weeks of follow-up in the exercise training compared to the controls. BNP demonstrated a relevant decrease at 17 weeks of follow-up when both groups were compared. Further, oxygen consumption per unit time per kg (VO\textsubscript{2/kg}) was slightly increased in the exercise training group compared to the controls.

Effects of regular exercise training on circulating progenitor cells: FACS-analysis showed that a symptom-limited (ischemic) exercise training led to a significant time-dependent increase from t\textsubscript{0} (2 weeks after acute AMI) to five weeks of follow-up in the CD45\textsuperscript{+}/CD34\textsuperscript{+} CPC-fraction from 257 ± 102 to 302 ± 128 cells per 1 million MNCs (p = 0.022) and in the CD45\textsuperscript{+}/CD34\textsuperscript{+} CPC-fraction from 64 ± 26 to 88 ± 46 cells per 1 million MNCs, reaching nearly the level of onset measurement. In the nonexercise training group the number of CPCs remained stable from t\textsubscript{0} to five weeks and 17 weeks of follow-up. The number of CD45\textsuperscript{+}/CD34\textsuperscript{+} cells counted 275 ± 68 cells per 1 million MNCs at t\textsubscript{0}, 254 ± 66 cells per 1 million MNCs five weeks of follow-up and 268 ± 69 cells per 1 million MNCs 17 weeks of follow-up (Fig. 5 a and Table 3). The number

| Clinical status | t\textsubscript{0} | t\textsubscript{1} | p\textsuperscript{+} | t\textsubscript{2} | p\textsuperscript{#} | p\textsuperscript{§} |
|-----------------|-------------------|-------------------|---------------------|-------------------|---------------------|---------------------|
| NYHA-classification | 2.64 ± 0.57 | 1.32 ± 0.48 | <0.001 | 1.52 ± 0.51 | 0.096 | <0.001 |
| exercise training controls | 2.75 ± 0.62 | 2.25 ± 0.62 | <0.001 | 2.42 ± 0.52 | n.s. | n.s. |
| BNP, pg/ml | 121 ± 94 | 75 ± 47 | <0.001 | 54 ± 42 | <0.001 | <0.001 |
| exercise training controls | 127 ± 78 | 111 ± 80 | n.s. | 92 ± 63 | n.s. | n.s. |
| Ergometry | 101 ± 33 | 137 ± 53 | <0.001 |
| max. workload, watt | 101 ± 33 | 137 ± 53 | <0.001 |
| exercise training controls | - | - | - |
| Duration of exercise, min | 5.68 ± 2.63 | 7.18 | 0.007 |
| exercise training controls | - | - | - |
| Exercise echocardiography | 46 ± 12 | 49 ± 12 | 0.028 | 49 ± 11 | n.s. | 0.009 |
| EF at rest, % | 48 ± 13 | 49 ± 16 | n.s. | 49 ± 12 | n.s. | n.s. |
| exercise training controls | - | - | - |
| Exercise spiroergometry | 57 ± 14 | 60 ± 14 | 0.031 | 60 ± 15 | n.s. | 0.020 |
| max. workload, watt | 57 ± 6 | 58 ± 7 | n.s. | 59 ± 8 | n.s. | n.s. |
| exercise training controls | - | - | - |
| VO\textsubscript{2max}, ml/min | 123 ± 47 | 140 ± 53 | 0.004 | 137 ± 52 | n.s. | 0.011 |
| exercise training controls | 125 ± 33 | 122 ± 41 | n.s. | 131 ± 44 | n.s. | n.s. |
| VO\textsubscript{2max/kg}, ml/min/kg | 1641 ± 522 | 1842 ± 724 | 0.017 | 1855 ± 695 | n.s. | 0.012 |
| exercise training controls | 1389 ± 547 | 1439 ± 465 | n.s. | 1851 ± 429 | 0.030 | n.s. |
| VO\textsubscript{2max/kg}, ml/min/kg | 20.0 ± 4.3 | 22.5 ± 6.5 | 0.015 | 22.9 ± 6.6 | n.s. | 0.012 |
| exercise training controls | 17.6 ± 5.6 | 17.3 ± 4.5 | n.s. | 21.7 ± 5.0 | 0.093 | n.s. |

EF = left ventricular ejection fraction; VO\textsubscript{2max} = maximum oxygen consumption; BNP = brain natriuretic peptide; Values are mean ± SD. p\textsuperscript{+} = t\textsubscript{0} vs. t\textsubscript{1}; p\textsuperscript{#} = t\textsubscript{1} vs. t\textsubscript{2}; p\textsuperscript{§} = t\textsubscript{0} vs. t\textsubscript{2}.

Table 2. Response to exercise training and traditionally rehabilitation in patients with STEMI evaluated by ergometry, exercise echocardiography, exercise spiroergometry and BNP (brain natriuretic peptide).
of CD45+/CD133+ cells was 73 ± 22, five weeks of follow-up 58 ± 19 and 17 weeks of follow-up 72 ± 23 cells per 1 million MNCs. In the exercise training group, the CD45+/CD133+ CPCs was slightly increased at five weeks of follow-up (after the physical training) compared to the controls (Fig. 5 b and Table 3).

### Effects of regular exercise training on CPC function

In order to assess the influence of regular exercise training on CPC function, the migration capacity of the CPCs was analyzed. Regular exercise training in patients with STEMI led to a significant enhancement of CPC migration capacity after the three weeks lasting training program from 113 ± 12 to 199 ± 57% (SDF-1, in relation to control cells without cytokine, p<0.001), and from 121 ± 19% to 242 ± 95% (VEGF, in relation to control cells without cytokine, p<0.001), although this phenomenon had vanished by three months thereafter (Fig. 4 and 6 a, b). The CPCs migration capacity was significantly increased after the physical training (t1) in the exercise training group compared to the controls.

The improved ability of the CPCs to migrate in the exercise training group was accompanied by a stable number of colonies, as demonstrated by CFU-erythroid and CFU-granulocyte/monocyte. In the non-exercise group after STEMI, the migration capacity revealed no increase over the investigated time, whereas the number of colonies in the clonogenic assays declined continuously after acute myocardial infarction from 23 ± 16 colonies before ET to 7 ± 7 colonies three months later in the CFU-GM assay and significantly from 84 ± 51 colonies before ET to 26 ± 29 colonies three months later in the CFU-E assay (Table 3).

### Discussion

In this prospective clinical trial, we assessed the effects of regular exercise training in patients with STEMI on clinical parameters (including BNP-level) and on the quantity and quality of CPCs. In patients with STEMI, regular symptom-limited exercise training over three weeks was able to (i) improve the clinical condition (NYHA-classification), (ii) increase the left ventricular ejection fraction at peak exercise, (iii) improve the cardiovascular capacity (exercise spiroergometry) and (iv) enhance the mobilization of blood-derived circulating...
Fig. 5. Quantitative evaluation of CD45+/CD34+ cells (Fig. 5a) and CD45+/CD133+ cells (Fig. 5b) by flow cytometry analysis. Patients with exercise training shows a temporarily increase of the CPCs between t0 and t1. In the control group (patients with traditional rehabilitation) were no differences observed. Values are expressed as total numbers of CD45+/CD34+ cells, respectively CD45+/CD133+ cells, related to 1x10^6 MNCs. Values are mean ± SD.

t0, before exercise training; t1, after exercise training; t2, 3 months after exercise training.

Fig. 6. Quantitative evaluation of migration capacity of CPCs in boyden chamber. Relative amount of migrated cells to SDF-1 (a) and VEGF (b) in comparison to cells without chemokines in blood samples of patients with STEMI and exercise training and patients with STEMI and traditional rehabilitation, blood samples were analyzed at 3 different time points. Values are expressed as % of migrated cells without chemokine. Values are mean ± SD.
CD133 is still unknown. Using CD133 expression to select BM-CPCs may contribute to the regeneration of ischemic myocardium and to differentiate into cardiac myocytes, increased in patients after acute myocardial infarction [12]. BM-CPCs have been shown to be significantly increased in an animal model [11]. In addition, human bone marrow-derived EPCs and that heterologous, homologous or autologous transplanted EPCs become incorporated into neovascularisation foci [9, 11, 20, 21]. Such results from animal models have also been confirmed in clinical settings. An increase in EPCs and a possible participation in neovascularisation have both been demonstrated in patients with acute myocardial infarction [12, 22]. Clinical trials studying the intracoronary transplantation of BM stem / progenitor cells as well as BM-derived autologous circulating progenitor cells have also shown that these cells have the potential to improve the function of the myocardium after acute ischemic injury [23-27]. Nevertheless, many questions remain to be answered.

Does a regular exercise training have any effect on the number and function of circulating progenitor cells after myocardial infarction? Circulating progenitor cells as CD45+/CD34+ and CD45+/CD133+ stem cells were investigated, both are progenitor cells that have the capacity to proliferate, migrate, and differentiate into various mature cell types [28, 29]. These bone marrow-derived circulating progenitor cells express unique surface markers, as CD34 and the early hematopoietic cell marker CD133 (AC133) [30, 31]. Tissue ischemia was found to mobilize endothelial progenitor cells into the peripheral blood and to contribute to neovascularisation in an animal model [11]. In addition, human BM-CPCs have been shown to be significantly increased in patients after acute myocardial infarction (AMI) [12] and to differentiate into cardiac myocytes, endothelial cells and smooth muscle cells following AMI [32]. Experimental studies suggested that BM-CPCs may contribute to the regeneration of infarcted myocardium [33] and improve neovascularisation of ischemic myocardium [35-38]. However, the function of early hematopoietic stem cell marker CD133 is still unknown. Using CD133 expression to define a very early subset of progenitor cells, Peschle et al. [31] isolated a CD133+ subpopulation of cells, which were able to differentiate into mature endothelial cells. This present study is the first report showing that regular symptom-limited exercise training is sufficient to trigger a time-dependent increase in circulating progenitor cells and their function in patients who had previously suffered an acute myocardial infarction.

Regular exercise training leads to a significant increase in circulating progenitor cells (CD34+ cells, respectively CD133+ cells). This augmentation in CPCs is comparable with the response to other therapeutic stimuli that increase CPCs, such as a four week long statin therapy [39] or VEGF-gene therapy [10, 40]. The transient increase in circulating progenitor cells after regular symptom-limited (ischemic and /or subischemic) exercise training reached a maximum after the exercise training, but did not persist up until three months after the regular exercise training. This finding extends our knowledge about the long-term effect of rehabilitation training programs on the changes in circulating progenitor cells. Laufs and colleagues described a significant increase in circulating progenitor cells after a 4 week, non-controlled rehabilitation training program in patients with stable coronary artery disease without exercise-induced ischemia [41]. In that trial it was speculated that asymptomatic tissue ischemia leads to an increase in vasculogenic cytokines, in the same way that symptomatic tissue ischemia does.

It is important to note here that an increase in CPCs is associated with significant increases in both CD34+ cells and CD133+ cells. This observation suggests that the response of the bone marrow to episodes of ischemia is not restricted to a specific cell type release, but is much rather a complex reaction where various progenitor cell populations and leukocyte subtypes become involved. Not only is the quantity of circulation progenitor cells altered, but their function is also modified by ischemic conditions and therapeutic interventions [42]. Migration of CPCs to ischemic tissue is believed to play a major role in the growth of new bloodvessels. Migration is essential for the ability of stem / progenitor cells to invade ischemic tissue. SDF-1 and VEGF are both profoundly upregulated in hypoxic tissue and may therefore represent physiologically relevant chemoattractants for the recruitment of circulating progenitor cells to ischemia sites [43, 44]. Indeed, intramuscular injection of SDF-1 has recently been shown to increase the number of incorporated endothelial progenitor cells and to improve neovascularisation in vivo [45].

In the present study, a migration assay was performed to investigate the ability of CPCs to migrate into ischemic tissue. The Boyden Chamber simulates the capacity of circulating progenitor cells to migrate to a chemotaxin such as VEGF or SDF-1 in patients with STEMI. This finding is consistent with the notion that both VEGF and SDF-1, which were differentially regulated during ischemic training, are involved primarily in altering the functional characteristics of CPCs. The result of the phenotypic analysis was not in accordance with a larger number of hematopoietic colonies obtained from peripheral blood mononuclear cells of patients with acute myocardial infarction performing regular exercise training, instead the number of the colonies remained stable over the investigated time. In patients with acute myocardial infarction performing no rehabilitation a continuous decrease in the number of hematopoietic colonies was assessed. This is in accordance with Massa et al [46], demonstrating a
continuous decline at 24 hours and 7 days with respect to admission with acute myocardial infarction. We sug-
gest that immediately after acute myocardial infarction the number of colonies goes up, but then decline con-
tinuously. If regular exercise training (rehabilitation) is performed after acute myocardial infarction the de-
cline of the number of colonies is delayed or even prevented. Improvement of clinical and hemodynamic param-
eters after regular exercise-training ET improves the quality of life in both men and women with mod-
erate, chronic heart failure [47]. Likewise, we also found a sig-
nificant difference in the left ventricular ejec-
tion fraction after peak exercise and the enhanced maxi-
um oxygen uptake (VO2 max) after a regular three week-long ET.

ET in chronic heart failure reduces catecholamines and vascular peripheral resistance while modulating heart rate variability and the baroreflex gain [51]. In a multicentre, randomised controlled clinical study (ELVD) in patients with a first Q wave myocardial in-
farction [52], regional wall motion abnormalities and endystolic volume both decreased 6 months after the ET. In that study it was suggested that an enhanced contractile performance predominantly contributed to the improved left ventricular function in their patients who showed an ejection fraction of below 40%.

Our findings show a significant increase in left ven-
tricular function and peak exercise both in the bicycle echocardiography after ET, similar to the data of Gi-
nannuzzi et al., but we did not find an increase in the left ventricular ejection fraction at rest. This difference may have arisen because of the lower left ventricular ejection fraction and the longer exercise program peri-
od (6 months) that was evident in the ELVD study. On the basis of the findings of Giannuzzi et al. [52], it is tempting to speculate that regular exercise training may decrease endystolic volume and regional wall motion abnormalities by bringing about beneficial changes in the autonomic balance and / or baroreflex gain. This is in line with an attenuation of the vaso-
constrictor influence (mainly due to reduced sympa-
thetic activity) and an increase in vagal tone [51].

Regular exercise training improved myocardial per-
fusion in stable coronary artery disease [53]. In acute myocardial infarction, long-term exercise training im-
proved left ventricular ejection fraction evaluated by radionuclide ventriculography [54]. Furthermore, early post-infarction cardiac rehabilitation improved the cardiorespiratory fitness with direct measurement of maximal oxygen uptake (VO2 max) [55].

In the present study, we demonstrate an increased exercise capacity, an improved cardiac function and a better cardiorespiratory condition in patients with acute myocardial infarction performing regular exer-
cise training. In an animal model physical training im-
proves the number of colonies and enhances angiogenesis [41]. One mech-
nism of repair by which physical training may im-
prove in human cardiac function after myocardial in-
farction, is considered to be the mobilization of bone marrow-derived progenitor cells homing into the dam-
aged tissue area, where they induced neovascularisation [56] [57]. Homing of the progenitor cells can be improved by the chemokine stem cell-derived factor 1 (SDF-1), which is intrinsically produced by the my-
ocardium after myocardial infarction. As recently shown, the intramyocardial delivery of SDF-1 increases the homing of c-kit+ stem cells [58]. The beneficial effect of regular exercise training on myocardial perfu-
sion has not been well established in humans. Howev-
er, increased myocardial perfusion was demonstrated after symptom-limited exercise training after acute my-
ocardial infarction [59] [60]. Mechanisms that promote enhanced myocardial perfusion include significant re-
lease of bone marrow-derived progenitor cells into circulation, as observed in our study. In addition, paracrine angiogenic factors, such as vascular endothelial growth factor are released after physical training and may increase neovascularisation in ischemic tissue [41] [61]. Enhanced neovascularisation may serve as one important mechanism facilitating reduction of in-
farct size after myocardial infarction.

In this first prospective clinical study, we were able to confirm the efficacy of a regular three week-long (15 times) symptom-limited (ischemia) exercise train-
ing in increasing both cardiac function and number of CPCs amongst patients with STEMI. The fol-
lowing major conclusions can be drawn from our find-
ings: (i) local tissue ischemia induced by symptom-lim-
ited exercise training enhance the mobilization of re-
generative progenitor cells from bone marrow into pe-
niipheral blood, (ii) ischemia may be involved in the regulation of the migration capacity of CPCs and (iii) enhanced mobilization and increase migration capacity of CPCs are associated with improved cardiac func-
tion and cardioprotective condition. Thus, early short term exercise training has beneficial effects for the re-
habilitation of patients with STEMI.

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