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Effects of aerobic interval training and continuous training on cellular markers of endothelial integrity in coronary artery disease: a SAINTEX-CAD substudy

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Van Craenenbroeck EM, Frederix G, Pattyn N, Beckers P, Van Craenenbroeck AH, Gevaert A, Possemiers N, Cornelissen V, Goetschalckx K, Vrints CJ, Vanhees L, Hoymans VY. Effects of aerobic interval training and continuous training on cellular markers of endothelial integrity in coronary artery disease: a SAINTEX-CAD substudy. Am J Physiol Heart Circ Physiol 309: H1876–H1882, 2015. First published October 9, 2015; doi:10.1152/ajpheart.00341.2015.—In this large multicenter trial, we aimed to assess the effect of aerobic exercise training in stable coronary artery disease (CAD) patients on cellular markers of endothelial integrity and to examine their relation with improvement of endothelial function. Two-hundred CAD patients (left ventricular ejection fraction > 40%, 90% male, mean age 58.4 ± 9.1 yr) were randomized on a 1:1 base to a supervised 12-wk rehabilitation program of either aerobic interval training or aerobic continuous training on a bicycle. At baseline and after 12 wk, numbers of circulating CD34+/KDR+/CD45dim endothelial progenitor cells (EPCs), CD31+/CD34+/CXCR4+ angiogenic T cells, and CD31+/CD42b+ endothelial microparticles (EMPs) were analyzed by flow cytometry. Endothelial function was assessed by flow-mediated dilation (FMD) of the brachial artery. After 12 wk of aerobic interval training or aerobic continuous training, numbers of circulating EPCs, angiogenic T cells, and EMPS were comparable with baseline levels. Whereas improvement in peak oxygen consumption was correlated to improvement in FMD (Pearson r = 0.17, P = 0.035), a direct correlation of baseline or posttraining EPCs, angiogenic T cells, and EMP levels with FMD was absent. Baseline EPC levels related inversely to the magnitude of the increases in peak oxygen consumption (Spearman rho = −0.245, P = 0.027) and FMD (Spearman rho = −0.374, P = 0.001) following exercise training. In conclusion, endothelial function improvement in response to exercise training in patients with CAD did not relate to altered levels of EPCs and angiogenic T cells and/or a diminished shedding of EMPs into the circulation. EMP flow cytometry may be predictive of the increase in aerobic capacity and endothelial function.

Exercise training; endothelial progenitor cells; endothelial microparticles; coronary artery disease

NEW & NOTEWORTHY

Three months of exercise training in stable coronary artery disease patients did not alter cellular markers of endothelial integrity (endothelial progenitor and angiogenic T cells, microparticles). The number of endothelial microparticles at baseline was an important within-patient predictor of the increases in aerobic capacity and peripheral endothelial function at training completion.

Exercise training is recognized as an important preventative and therapeutic strategy in cardiovascular disease, in part through its beneficial effects on peak oxygen consumption (peak VO2) and endothelial function (17, 27), both strong and independent prognostic markers in patients with coronary artery disease (CAD) (31, 40).

Endothelial dysfunction, clinically assessed by impaired vasodilator response to various stimuli, is an important marker of preclinical atherosclerosis. It is a systemic characteristic in patients with CAD and simultaneously affects both coronaries and peripheral arteries (1). Impaired endothelial-dependent vasodilation of coronary arteries leads to reduced myocardial perfusion and impaired ventricular function. At the other hand, impaired vasodilatory capacity of peripheral vessels limits the peripheral nutritive blood flow to skeletal muscles and increases systemic vascular resistance and afterload. The clinical importance of these processes are mirrored in the strong relation between endothelial-dependent vasodilation and peak VO2 (14). In view of the fundamental impact on morbidity and mortality in CAD, restoring dysfunctional endothelium has become a major therapeutic goal.

Small single center studies show that peak VO2 increases to a larger extent with aerobic interval training (AIT) at higher intensity compared with moderate intensity continuous training.
in patients with metabolic syndrome, after acute coronary syndrome, following coronary artery bypass surgery and in patients with ischemic heart failure (3, 26, 28, 43, 44). Interestingly, in the latter patient group, as well as in patients with metabolic syndrome, AIT also led to a significantly larger improvement in peripheral endothelial-dependent vasodilation (36, 44). The reason for the superior effect of AIT on peripheral endothelial function is not completely understood. It is, however, conceivable that differences in vascular shear stress patterns between exercise protocols induce different molecular and cellular responses.

Indeed, the enhanced production of nitric oxide in response to shear stress plays a critical role in the beneficial effects of exercise training on endothelial function. In addition, the anti-inflammatory, free radical reducing and permeability decreasing properties of exercise may all contribute to improvement of endothelial function (13).

It has also been suggested that endothelial progenitor cells (EPCs) could add to these favorable changes (23). EPCs are a rather small population of cells that can be mobilized from the bone marrow into the peripheral blood by various stimuli, such as ischemia and chemokines. They participate in the repair of endothelial damage, thereby possibly influencing endothelial function (23). EPC function is regulated by angiogenic T cells that express the platelet endothelial cell adhesion molecule (CD31) and the receptor for stromal-derived factor 1 (CXCR4) (20). Thus far, because of small-scale, single-center trials, heterogeneity in patient populations and inconsistency of study results, it was not possible to draw firm conclusions on a role for EPCs and angiogenic T cells as mediators of the training-induced improvement of endothelial function in CAD (11).

The main objective of the Study on Aerobic Interval Exercise Training in CAD (SAINTEX-CAD) study was to investigate whether a 12-wk program of AIT yields a larger gain in peak VO2 and endothelial function compared with a similar training program of aerobic continuous training (ACT) (9). The results of this large, randomized, multicenter study involving 200 patients with CAD demonstrated that AIT and ACT are equal in improving peak VO2 and peripheral endothelial function (8). The purpose of this substudy from SAINTEX-CAD is to investigate whether aerobic exercise training (AIT vs. ACT) can mobilize EPCs and other related cellular blood markers of endothelial integrity and to examine the relationship between these blood markers and the improvement of endothelial function.

METHODS

Patients and Study Design

A detailed description of the rationale and design of the SAINTEX-CAD study has been previously published (9). Briefly, 200 stable patients with cardiovascular disease were enrolled at the cardiac rehabilitation centers of the University Hospitals of Antwerp (Center 1, n = 100) and Leuven (Center 2, n = 100), Belgium, between October 2011 and April 2013. The main study inclusion criteria were 1) angiographically documented CAD or previous acute myocardial infarction (AMI); 2) left ventricular ejection fraction > 40%; 3) optimal medical treatment; 4) stable with regard to symptoms and medication for at least 4 wk; and 5) included between 4 and 12 wk following AMI, elective percutaneous coronary intervention (PCI), or coronary artery bypass grafting (CABG). Patients were randomized to either aerobic interval training (AIT) or aerobic continuous training (ACT) on a 1:1 base by an online protocol at Center 1. At baseline and after 3 mo, patients underwent cardiopulmonary exercise testing, vascular function assessment, and blood sampling. Assessments and blood sampling took place in the week before the first training session and between day 3 and 7 after the last training session. Blood sampling was performed in the morning, in fasting conditions, and patients refrained from exercise at least 8 h before the measurements.

The Laboratory of Cellular and Molecular Cardiology of Center 1 served as the central core laboratory responsible for the EPCs and angiogenic T-cell analyses of both centers.

The study complies with the Declaration of Helsinki and was approved by the Local Ethics Committees, and written informed consent was obtained from each participant.

Exercise Training

Thirty-six supervised exercise sessions were implemented at a rate of three sessions a week during 12 wk. Patients exercised on a bicycle; exercise load was adjusted to comply with the target heart rate (HR) throughout the 12-wk training period. The target HR was calculated using the initial cardiopulmonary exercise test. A new cardiopulmonary exercise testing was performed after 6 wk to adjust the training intensity according to the achieved peak HR. Warm-up and cool-down HR were excluded from mean HR calculations. Patients randomized to the AIT group cycled during 38 min in four 4-min intervals at 90–95% of peak HR (Fig. 1). Each interval was separated by 3-min

Fig. 1. Schematic illustration of both training programs.
active pauses, cycling at 50–70% of peak HR. The session started with a 10-min warm-up and ended with a 3-min cool-down. Patients in the ACT group continuously cycled at an intensity of at least 70–75% of peak HR during 37 min. The session started with a 5-min warm-up and ended with a 5-min cool-down.

Clinical Assessments

Cardiopulmonary exercise testing. Cardiopulmonary exercise testing was performed at baseline and after 12 wk using an individualized cycle ergometer ramp protocol (20 W + 20 W/min or 10 W + 10 W/min). Breath-by-breath gas exchange measurements allowed online determination of ventilation, oxygen uptake (VO\(_2\)) and carbon dioxide production every 10 s. Peak VO\(_2\) was determined as the mean value of three measures of VO\(_2\) during the final 30 s of exercise. The anaerobic threshold, assessed by the V-slope method (2), and the respiratory exchange ratio were recorded.

Endothelium-dependent vasodilation.. All analyses were performed in the morning, in fasting conditions and in a quiet temperature-controlled room (21–24°C), by a trained operator that was blinded for the study intervention. Subjects refrained from exercise, food, and caffeine at least 8 h before the measurements. Blood pressure was obtained after 10 min of rest with an automated blood pressure monitor. Endothelial function was assessed by flow-mediated dilation (FMD) of the brachial artery using ultrasound (Center 1, AUS Ultrasound System, Esaote; Center 2, GE Healthcare, Vivid 7), according to international guidelines (10, 35). A high-resolution linear-array vascular probe was used (Center 1, 10 MHz; Center 2, 5–13 MHz). Patients were positioned supine with the right arm resting on an arm support; the brachial artery was imaged above the antecubital fossa in the longitudinal plane. After a recording of the baseline diameter for at least 1 min of stable distension waveforms, a blood pressure cuff on the forearm was inflated to at least 200 or 60 mmHg higher than the resting systolic blood pressure, according to the recent guidelines by Thijssen et al. (35). Images were continuously recorded before cuff inflation for 1 min and after cuff deflation for at least 3 min. Endothelium-independent vasodilation was measured after administering 1 dose (0.4 mg) of nitroglycerine (Nitrolingual Pumpspray) sublingually (nitrate-mediated dilation). Images were continuously recorded from the 3rd until the 9th min after administering nitroglycerine. Images were analyzed using edge-detection software FMD-i by Flomedi (Flomedi, Brussels, Belgium). FMD was expressed as the change in poststimulus diameter as a percentage of the baseline diameter. Analyses were blinded in both study centers.

Flowcytometric Quantification of Cellular Markers of Endothelial Integrity

Quantification of EPCs. EPCs were defined as CD34\(^+\) KDR\(^+\) CD45\(_{dim}\) cells (32). Fixed whole blood (TransFix, Caltag Medsystems, Buckingham, UK) was processed 2 to 3 days after sampling (19). Red blood cells were lysed using ammonium chloride solution (NH\(_4\)Cl). After pretreatment with Fc receptor blocking reagent (Miltenyi Biotec, Bergisch Gladbach, Germany), samples were stained with the following antibodies: CD34-PE-Cy7 (BD Pharmingen, Erembodegem, Belgium), KDR-APC (R&D Systems, Minnesota), and CD45-APC-H7 (BD Pharmingen). Negative controls included fluorescence-minus-one samples and unstained samples. The nucleic acid dye SYTO 13 (Life Technologies, Gent, Belgium) allowed exclusion of nonnucleated cells and cellular debris. At least one million total events were recorded on a FACScantio II flow cytometer (Becton Dickinson, New Jersey). Numbers of EPCs were analyzed using FACSDiva software (Becton Dickinson, version 6.1.2) and expressed as cells per million CD45\(_{+}\) mononuclear cells with low forward (FSC) and side scatter (SSC). Briefly, after exclusion of cellular aggregates (FSC area vs. height plot) and debris (SYTO 13 negative), a primary gate was set on the mononuclear cells. A second gate was next set on a CD45 versus SSC dot plot to contain all CD45\(_{dim}\) events, as previously described (32). CD34\(^+\) and KDR\(^+\) events were analyzed in this population.

Quantification of angiogenic T cells. Angiogenic T cells were defined as CD31\(^+\) CD3\(^+\) CXCR4\(^+\) cells (20). After red cell lysis and Fc receptor blocking at days 2 or 3, fixed whole blood was stained with CD31-FITC, CD3-PerCP, and CXCR4-APC antibodies (all from BD Biosciences). Unstained samples and fluorescence-minus-one samples for CD31 and CXCR4 were used as controls. At least 500,000 total events were analyzed using FACSDiva software and expressed as cells per million mononuclear cells. Doublets and aggregates were excluded by selecting single cells on a FSC area versus FSC height plot.

Quantification of endothelial microparticles. Endothelial microparticles (EMPs) were defined as CD31\(^+\) CD42b\(^-\) particles smaller than 1 \(\mu\)m (Fluoresbrite YG 1-\(\mu\)m calibration size beads, Polysciences, Eppelheim, Germany). EMP enumeration was performed only on samples collected at Center 1 (n = 90). For this purpose, platelet poor plasma was produced immediately after blood sampling by double centrifugation at 1550 g. Antibodies used were CD31-PE and CD42b-FITC (both from BD Biosciences). Samples were analyzed as we previously described, enabling the evaluation of circulating EMPs per microliter platelet poor plasma (39).

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

Statistical Analysis

Continuous data are expressed as means ± SD. Skewed distributed data (1-sample Kolmogorov-Smirnov test) are presented as median (range). Baseline comparisons were performed using independent sample t-test or \(\chi^2\)-test where appropriate. Differences over time between groups (= interaction) were analyzed by univariable two-way repeated measures analysis of covariance (ANCOVA) with age and pathology as covariates. Whereas no center-effect was found for peak VO\(_2\), FMD values were significantly higher in center 2. Therefore, ANCOVA for FMD included age, pathology, and center as covariates. Percent changes of FMD were skewed and therefore expressed as median (range). Pearson or Spearman correlation coefficients were used for correlations. A stepwise multiple linear regression analysis was used to assess independent determinants of peak VO\(_2\) changes with adjustment for significant determinants on correlation analysis. All tests were two-sided, and a P value of 0.05 was considered statistically significant. All analyses were performed using SPSS 20.0 (SPSS, Chicago, IL).

RESULTS

PATIENT CHARACTERISTICS

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

Baseline Levels of Endothelial Integrity Markers

Numbers of circulating EPCs, angiogenic T cells, and EMPs were not associated with the presence of cardiovascular risk factors like hypertension, diabetes, and smoking (all, \(P > 0.05\)). In addition, numbers did not correlate with age, hs-CRP or eGFR (all, \(P > 0.05\)). There were no differences in EPCs,
Table 1. Demographic and clinical characteristics at baseline

<table>
<thead>
<tr>
<th></th>
<th>AIT</th>
<th>ACT</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>57.0 ± 8.8</td>
<td>59.9 ± 9.2</td>
<td>0.023</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>91/9</td>
<td>89/11</td>
<td>NS</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>57.1 ± 8.5</td>
<td>56.8 ± 7.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

Cardiovascular risk

| Body mass index, kg/m² | 28.0 ± 4.4 | 28.5 ± 4.3 | NS       |
| Diabtes, %             | 20          | 18         | NS       |
| Hypertension, %        | 58          | 46         | NS       |
| Smoking, %             | 73          | 74         | NS       |

Laboratory measurements

| Hemoglobin, g/dl       | 14.3 ± 1.3 | 14.2 ± 1.4 | NS       |
| Leukocytes, cells/μl   | 6.698 ± 1.575 | 6.570 ± 2.094 | NS |
| eGFR, ml·min⁻¹·1.73 m⁻² | 78.0 ± 12.9 | 77.5 ± 12.6 | NS       |
| hs-CRP, mg/l           | 4.71 ± 9.4 | 3.45 ± 7.2 | NS       |

Exercise Capacity

| Peak V̇O₂, ml·kg⁻¹·min⁻¹ | 23.3 ± 5.8 | 22.5 ± 5.6 | NS       |
| % V̇O₂ predicted         | 82.8 ± 22.6 | 83.3 ± 22.7 | NS       |
| Maximal workload, W      | 152 ± 39   | 144 ± 41   | NS       |

Medication, %

| Statin                 | 97          | 99         | NS       |
| Aspirin                | 97          | 95         | NS       |
| β-Blocker              | 84          | 83         | NS       |
| ACE inhibitor/ARB      | 77          | 72         | NS       |
| Insulin therapy        | 5           | 4          | NS       |

Values are means ± SD or percentage. NS, not significant; M, male; F, female; LVEF, left ventricular ejection fraction; CABG, coronary artery bypass graft; PCI, percutaneous coronary intervention; AMI, acute myocardial infarction; eGFR, estimated glomerular filtration rate; hs-CRP, high sensitivity C-reactive protein; V̇O₂, oxygen uptake; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker.

angiogenic T cells, and EMP numbers between post-AMI, post-PCI or post-CABG patients (all, P > 0.05).

Changes in Clinical Parameters Posttraining

As previously reported, mean training intensity was 88% of peak HR in the AIT group and 80% of peak HR in the ACT group (8). Peak V̇O₂ was significantly higher in both groups after 12 wk (AIT, 22.7 ± 17.6% vs. ACT, 20.3 ± 15.3%; p-time < 0.001, p-interaction = 0.87, Table 2). In addition, FMD improved significantly in both groups [AIT, median 34.1% (range −69.8% to 646%) and ACT median 7.14% (range, −66.7% to 503%); p-time < 0.001, p-interaction = 0.95]. The improvement in peak V̇O₂ was correlated to improvement in FMD (Pearson r = 0.17, P = 0.035). Improvements in both outcomes were comparable for both training interventions.

Changes in Numbers of EPCs, Angiogenic T Cells, and EMP Posttraining

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

Cellular Markers as Predictors of Training-Induced Response

At baseline, no correlations were observed between cellular markers and endothelial function or aerobic capacity. However, baseline numbers of EMPs were related to the magnitude of the change in peak V̇O₂ (spearman rho = −0.245, P = 0.027) and the change in FMD (spearman rho = −0.374, P = 0.001) following exercise training (Fig. 2). For this analysis, the total cohort was pooled (AIT and ACT) since an interaction term was absent and EMP numbers were logarithmically transformed. These relations were maintained in multivariate regression analysis of logarithmically transformed EMP numbers and after correction for baseline peak V̇O₂ and age, variables that were related to the change in peak V̇O₂ in univariate analysis (β = −0.263, P = 0.01). Baseline EPCs or angiogenic T cells did not correlate with changes in FMD or peak V̇O₂.

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

<table>
<thead>
<tr>
<th></th>
<th>AIT 0 wk</th>
<th>AIT 12 wk</th>
<th>ACT 0 wk</th>
<th>ACT 12 wk</th>
<th>P Value</th>
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<tr>
<td>Exercise capacity</td>
<td></td>
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<tr>
<td>Peak V̇O₂, ml·kg⁻¹·min⁻¹</td>
<td>23.5 ± 5.7</td>
<td>28.6 ± 6.9</td>
<td>22.4 ± 5.6</td>
<td>26.8 ± 6.7</td>
<td>&lt;0.001 0.14 0.87</td>
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<tr>
<td>Endothelial function</td>
<td></td>
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<td>Baseline diameter, mm</td>
<td>3.96 ± 0.56</td>
<td>4.00 ± 0.50</td>
<td>3.93 ± 0.56</td>
<td>3.99 ± 0.65</td>
<td>0.50 0.80 0.77</td>
</tr>
<tr>
<td>Peak diameter, mm</td>
<td>4.19 ± 0.57</td>
<td>4.27 ± 0.54</td>
<td>4.12 ± 0.58</td>
<td>4.22 ± 0.67</td>
<td>0.23 0.52 0.78</td>
</tr>
<tr>
<td>FMD, %</td>
<td>5.26 ± 3.02</td>
<td>6.47 ± 2.79</td>
<td>5.61 ± 2.36</td>
<td>6.68 ± 3.09</td>
<td>&lt;0.001 0.26 0.95</td>
</tr>
<tr>
<td>Post-GTN diameter, mm</td>
<td>4.79 ± 0.57</td>
<td>4.85 ± 0.56</td>
<td>4.76 ± 0.66</td>
<td>4.74 ± 0.58</td>
<td>0.58 0.28 0.68</td>
</tr>
<tr>
<td>NMD, %</td>
<td>22.6 ± 6.57</td>
<td>22.2 ± 7.19</td>
<td>22.1 ± 7.3</td>
<td>22.1 ± 6.92</td>
<td>0.97 0.65 0.97</td>
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<tr>
<td>Endothelial integrity markers</td>
<td></td>
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<tr>
<td>EPCs/10⁶ MNCs</td>
<td>8.2 (0–51)</td>
<td>7.4 (0–53)</td>
<td>9.5 (0–37)</td>
<td>10.6 (0–106)</td>
<td>0.71 0.02 0.98</td>
</tr>
<tr>
<td>Angiogenic T cells/10⁶ MNCs</td>
<td>1.901 (186–20.494)</td>
<td>1.950 (117–26.247)</td>
<td>2.744 (132–24.000)</td>
<td>4.765 (164–31.392)</td>
<td>0.33 0.15 0.27</td>
</tr>
<tr>
<td>EMPs/μl</td>
<td>129 (47–756)</td>
<td>192 (47–755)</td>
<td>227 (80–715)</td>
<td>260 (60–922)</td>
<td>0.97 0.06 0.37</td>
</tr>
</tbody>
</table>

Values are means ± SD or median (range). Endothelial progenitor cells (EPCs), angiogenic T cells, and endothelial microparticles (EMPs) data were log transformed before analysis. ANCOVA with age and pathology as covariates was performed to test time and interaction effects. AIT, aerobic interval training; ACT; aerobic continuous training; FMD, flow-mediated dilation; GTN, glyceryl trinitrate; NMD, nitrate-mediated dilation; MNC, mononuclear cells.
DISCUSSION

To our knowledge, the current substudy of the SAINTEX-CAD trial is the largest randomized, multicenter study evaluating the effect of exercise training on cellular blood markers of endothelial integrity in stable CAD patients. Despite a significant improvement in peripheral endothelial function, we found no meaningful changes in the numbers of circulating EPCs, angiogenic T cells, and EMPs after 12 wk of AIT or ACT. EMP counts at baseline, however, were related to the improvements in peak $\dot{V}_O_2$ and FMD at completion of the training program.

Blood-Related Markers of Endothelial Damage and Repair

Endothelial dysfunction precedes overt atherosclerosis by many years and is an independent prognostic marker of cardiovascular events (30). Disruption of endothelial homeostasis results from imbalances in the production of nitric oxide and reactive oxygen species, local and systemic low-grade inflammation, and loss of endothelial cells by apoptosis (7). The injury of the vessel wall leads to the recruitment of circulating EPCs and angiogenic T cells to the site of endothelial disruption. It is well known from human and animal studies that EPCs and angiogenic T cells, which stimulate EPC function, actively participate in the repair of damaged endothelium (41, 42). Moreover, endothelial repair seems to improve with their increased numbers in the circulation, reflecting a higher regenerative capacity. Recently, joint efforts have led to the standardization of techniques for EPC enumeration, and consensus exists now on the phenotype of EPCs, defined as CD34$^+$KDR$^+$CD45$^{dim}$ cells (32, 38). This phenotype is considered to describe the true EPCs. They spontaneously form blood vessels in vivo and participate in endothelial repair in vitro and in vivo (24, 33, 41).

High levels of EMPs affect the endothelial cell layer lining in a negative manner, in contrast to EPCs and angiogenic T cells. EMPs are shed from the plasma membrane of endothelial cells upon their activation, apoptosis, or injury (18). These small particles may contribute to worsening of endothelium injury by impairing the endothelium-dependent vasodilation and modulating inflammation via leukocyte activation and transendothelial migration (12).

Impact of Exercise Training on Endothelial Function and Endothelial Repair

This is the first study to examine the influence of exercise training on circulating levels of EMPs and angiogenic T cells in patients with CAD. Data regarding the impact of physical training on the number of EPCs, however, are conflicting. One recent study reported an increase in CD34$^+$KDR$^+$CD45$^{dim}$ EPCs at the completion of a 4-wk aerobic exercise training program in 61% of stable patients with a previous AMI ($n = 112$) (6). In that study, patients in the lowest tertile of baseline hs-CRP were most likely of obtaining an increase in EPCs. Variations in peak $\dot{V}_O_2$ were correlated with variations in EPCs, and patients without an increase in peak $\dot{V}_O_2$ ($n = 26$) demonstrated a lower improvement in EPC number compared with patients with an increase in peak $\dot{V}_O_2$. Likewise, Ikeda et al. (21) described that a 3-mo walking program of $>4$ h walking per week led to a gain in aerobic capacity and CD34$^+$CD133$^+$ EPC number in patients with recent AMI ($n = 23$). Moreover, Steiner et al. (34) provided evidence supporting a role for increased CD34$^+$KDR$^+$CD133$^+$ EPCs in the augmentation of endothelial function during exercise in patients with CAD ($n = 20$). Three months of aerobic exercise training resulted into a higher EPC level, which was positively correlated with the change in FMD. Paul et al. (29) also reported a rise in CD133$^+$KDR$^+$ EPCs in 35 out of 46 patients with CAD after 3 mo of aerobic exercise training (29). Brachial artery FMD, however, was not improved and did not correlate with the number of EPCs. In addition, the reduction in plasma hs-CRP was modest and did not reach statistical significance at program completion. Finally, Luk et al. (25) ($n = 32$) and Hansen et al. (15) ($n = 47$) did not find a significant increase in CD34$^+$KDR$^+$ EPCs in patients with CAD after 8 and 6 wk of aerobic exercise, respectively (15, 25). Although we must acknowledge the considerable variation in EPC phenotypes, our results are more consistent with these latter studies, indicating that EPCs, angiogenic T cells, and EMPs are not critically involved to the training-induced improvement of endothelial function in patients with CAD.

There are various potential reasons why no significant changes were found in the present study compared with some previous studies. Variations in the definition and the laboratory
protocols for EPC and EMP detection certainly complicate the comparison of interlaboratory results. The heterogeneity in exercise training protocols (including intensity, frequency, and duration) also could affect cell numbers. Furthermore, the time of blood sampling is of importance; EPC counts can be increased until 24 h after exercising. The time period between the training session and the blood sampling, however, is not always reported and might also introduce variability. Therefore, further research is needed to understand why and when cell numbers change in the setting of exercise training.

Responders to Exercise Training

To date, it is widely recognized that the individual response to exercise training in terms of aerobic capacity is highly variable among patients. Aerobic capacity is one of the strongest prognosticators in cardiovascular disease, but 20% of patients have a low or absent response in peak VO2 to training (4). The mechanisms driving this variability are not well understood, nor do we have good predictors of the response to exercise therapy. Heritability accounts for 45–50% of the anticipated effect of exercise training (5). In the present study, EMP count at baseline was an important within-patient predictor of the change in peak VO2 and peripheral endothelial function at completion of a 3-month training program. EMPs are considered as a marker of overall endothelial health (12). As the beneficial effects of exercise training on peak VO2 rely partly on improving endothelial-dependent vasodilation (13), patients with higher EMP levels at baseline, hence worse general endothelial health, could be less sensitive to the effect of exercise training. As such, the number of circulating EMPs holds promise as marker of response to exercise training. Although additional studies are needed to confirm its predictive value, EMP flow cytometry may offer guidance to clinicians and physiotherapists to tailor exercise protocols to the need of individual patients and thereby maximize the beneficial effects.

Limitations

The definition of EPCs is still a matter of debate. In our study, EPC enumeration was performed according to the recommendations of Van Craenenbroeck et al. (38). The phenotypic profile of EMPs may change according to the type of vascular injury (activation or apoptosis). It is therefore unlikely that our set of markers sufficiently labeled the entire EMP population. A final limitation is that we could not include a functional analysis of circulating angiogenic cells in this study design. Circulating angiogenic cells contribute to endothelial repair in a paracrine fashion (22), and reduced functional capacity is correlated with impaired endothelial dependent vasodilation (16). In previous work, we found that exercise induces favorable effects on the functional capacity of these cells in chronic heart failure patients (37).

Conclusions

Our results demonstrate that the improvement of endothelial function in response to exercise training in stable patients with CAD is neither related to altered levels of EPCs and angiogenic T cells nor to a diminished shedding of EMPs into the peripheral circulation. EMP counts at baseline, however, may be predictive of the extent of increase in aerobic capacity and endothelial function at completion of the training program.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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