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Original Article

Effects of aerobic and strength training on aerobic capacity, muscle strength, and gene expression of lymphomonocytes in patients with stable CAD

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Abstract: This study examined the effectiveness, suitability, and safety of a mixed interval-type aerobic and strength training program (MIAST) on physical fitness in patients with stable coronary artery disease (CAD) without history of myocardial infarction (MI). Twenty-three patients with stable CAD were randomly assigned to a MIAST (n = 12; mean age 58.6 years) or control (n = 11; 63.3 years) group. The MIAST group participated in the progressive training program twice a week for 21 weeks. Peak oxygen uptake (VO_{2peak}), workload, and exercise time were measured as were maximal muscle strength, serum lipids, glucose concentration, and the cross-sectional area (CSA) of knee extensors. The safety and suitability of the program were assessed by wireless electrocardiogram (ECG) monitoring and exercise diaries. VO_{2peak} (6.9%; $P < 0.05$) and exercise time (11.2%; $P < 0.05$) improved significantly after 12 weeks of training in the MIAST group compared to the control group. Muscle strength (19.9%; $P < 0.05$) and CSA (2.2%; $P < 0.05$) increased, and serum lipids and blood glucose tended to decrease after the training. The successful training program (increase in maximal oxygen uptake) increased the gene expression of oxygen metabolism and decreased the gene expression of inflammation pathways in lymphomonocytes. The MIAST program, including interval-type aerobic and strength training, was safe, did not cause any adverse effects, and led to significant improvements in physical fitness in patients with stable CAD.

Keywords: Coronary heart disease, physical fitness, oxygen consumption, endurance training, resistance training

Introduction

Regular exercise has been shown to be an effective tool in the prevention and rehabilitation of coronary artery disease (CAD) [1, 2]. It targets several risk factors [3, 4] and can be used safely in rehabilitation after myocardial infarction (MI). Exercise recommendations for healthy people [5] and patients with CAD [6] include both aerobic and strength training in a weekly training program. As both aerobic and strength training are recommended to patients with CAD, one possibility is to combine these two training modes and thus try to reap the benefits of both as effectively and safely as possible. The current knowledge about the benefits of combining resistance and aerobic training compared to control or simply aerobic training has been presented in a systematic review and meta-analysis of randomized controlled trials in patients with CAD [7].

Cardiac patients have traditionally undergone steady-state aerobic training [6], but several studies have also examined the effectiveness of interval training in increasing the aerobic capacity of CAD patients [8-13]. These studies have hypothesized that greater or similar

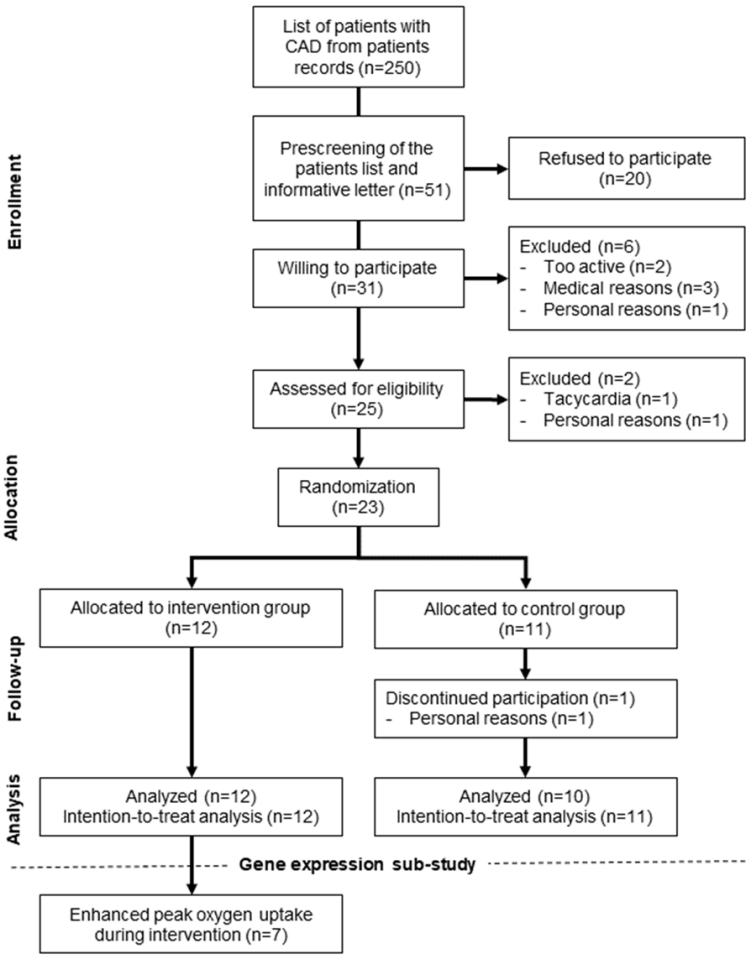

Figure 1. The flow chart of the study arrangement shows a number of subjects involved in enrollment, allocation, and follow-up, and finally in the gene-expression sub study. From the 250 CAD patients identified from the records of Central Hospital of Central Finland 23 were allocated to intervention or control group. Subjects were excluded due to not fulfilling the selection criteria, fulfilling the exclusion criteria, or refusing to participate. Seven subjects who enhanced their peak oxygen up-take during the study intervention were included in the gene expression sub-study.

improvements in aerobic capacity could be achieved more quickly through interval training compared to moderate-intensity steady-state training. As expected, high-intensity interval training (HIIT) has proved better than moderate-intensity continuous training (MCT) in improving patients' maximal aerobic capacity (VO_{2peak}), but the two methods yield no different effects on serum glucose, triglycerides, or HDL levels, but MCT may be better for reducing body weight and heart rate [13]. In additi0 on, physical activity may influence low-grade inflammation and lymphocyte function, observed as changes in blood cell gene expression [14, 15]. Several systemic disease states, including cardiovascular diseases (CVD), are reflected in the transcriptome and in functional changes in lymphomonocytes [16, 17].

The purpose of this pilot study was to examine the effectiveness and suitability of a mixed interval-type aerobic and strength training program (MIAST) performed twice a week on aerobic capacity, muscle strength and composition, and cardiovascular risk factors in patients with stable CAD but no history of MI. In a sub-study, we also investigated the effect of the training program on lymphomonocyte gene expression among the MIAST intervention participants who were able to increase their maximal oxygen uptake during the training program. The purpose of this sub-study was to identify the most up/down-regulated gene sets related to the training to increase our knowledge of the mechanisms mediating the exercise-induced effects.

Materials and methods

Design

We conducted a 21-week randomized controlled trial with a parallel-group design. Sedentary persons with stable CAD were randomly allocated in equal numbers to either a mixed aerobic and strength training (MIAST, n = 12) group or a control (n = 11) group (**Figure 1**) and were stratified by sex. Randomization was performed using randomized block design with a block size of 10 for males and of 4 for females. A statistician performed the randomization, and a researcher assigned participants to each group according to the randomization. All outcomes were measured as blinded. All participants were measured at baseline and after 12 weeks and 21 weeks of training. The study was designed according to the Declaration of

Helsinki and approved by the Ethics Committee of the Central Hospital of Central Finland. All participants provided written informed consent before inclusion in the study.

Participants

Patients with CAD who had undergone coronary angiography more than three months but not more than 18 months before the beginning of the study were identified from the patient records of the Central Hospital of Central Finland. From those 250 patients with CAD, an informative letter describing the study was sent to 51 patients who fulfilled the selection criteria. The inclusion criteria were as follows: 35-70 year old man or woman with stable CAD; 30-90% coronary artery stenosis in a maximum of two native coronary arteries diagnosed with coronary angiography; and a history of angina pectoris of class I to II according to the criteria of the Canadian Cardiovascular Society. Subjects were excluded when their treatment had been conservative or when angioplasties, coronary artery by-pass grafts, or stentings had been performed or when they had any of the following conditions: drug-eluting stents and transmural (Q-wave) MI in the left ventricle wall, unstable angina, a history of MI, arrhythmias, and conduction disorders, reduced left ventricular systolic function (ejection fraction < 40%), heart failure, significant valvular heart diseases, uncontrolled hypertension, stroke within the previous 6 months, chronic obstructive pulmonary disease (COPD), renal insufficiency, neurological disorders or diseases, anemia, active cancer, insulin-dependent diabetes, untreated hyper/hypothyreosis, exercise limiting hip, knee, or back disorders, rheumatoid arthritis, body mass index (BMI) ≥40, or serious mental disorders. They were also excluded if they had participated in a supervised systematic exercise training within one year before the beginning of the study.

Thirty-one patients agreed to participate in the study. Six were excluded based on the selection criteria. Therefore, 25 patients were assessed for eligibility. During the baseline measurements before randomization, one patient was excluded due to displaying ventricular tachycardia in the exercise stress test and another was excluded for personal reasons. Of the initial 23 patients, 22 completed the study (see the Results section).

Exercise stress test

The primary outcome was a change in peak oxygen uptake (VO_{2peak}; $ml^{-1}/min^{-1}/kg$). A symptom-limited exercise stress test was performed on an Ergoselect 100P stationary cycle (Ergoline GmbH, Bitz, Germany). One physician supervised the tests at baseline, and one of two other physicians supervised the tests at weeks 12 and 21. The test was preceded by a two-minute warm-up at the intensity of 20 W. The first test load was 50 W, and the load was increased by 25 W at each two-minute interval. The patients were monitored continuously throughout the test using a 12-lead electrocardiograph (CardioSoft® Version 5, GE Medical Systems Information Technologies GmbH, Freiburg, Germany). Heart rate (beats/min), workload (Watts; W), and each patient's rating of perceived exertion (Borg 6 to 20 scale) were recorded at the end of every load. Blood pressure was measured in a sitting position before the test, during the second minute of each stage, and during the five minutes of recovery. Oxygen uptake was measured and monitored with a Jaeger Oxycon Pro metabolic cart/gas analyzer (Viasys Healthcare GmbH, Höchberg, Germany) using a breath-by-breath-method. The patients exercised until they could no longer continue, were unable to maintain a pedaling frequency between 60-70 rpm, or had achieved a respiratory exchange ratio of more than 1.1, or until clinical criteria for test termination were observed. The VO_{2peak} was defined as the highest VO_2 obtained during the test.

Maximal isometric muscle force

The maximal isometric unilateral force of the right knee extensors was measured using a chair dynamometer (Metitur Ltd., Jyväskylä, Finland). The patient was in a seated position so that the hip and knee angles were 90° and 120°, respectively (with 180° meaning full knee extension). After three warm-up trials the patient was asked verbally to produce his or her maximal force as fast as possible in 3-5 seconds. A minimum of three maximal trials were measured, and the highest value was accepted as the result. A rest period between the trials lasted one minute.

Muscle composition and anthropometry

A computed tomography (CT) scan was obtained from the right thigh muscles using a So-

matom DR scanner (Siemens AG, Erlangen, Germany) before and after the intervention period. The scanning point was located mid-thigh, which was defined by palpation as the midpoint between the greater trochanter and the lateral joint line of the knee. CT scans were analyzed using a software program developed at our laboratory, BonAlyse 1.0 (BonAlyse Oy, Jyväskylä, Finland). The cross-sectional area (CSA) and lean tissue CSA (LCSA) were measured from the quadriceps femoris (QF) [18]. Body weight (kg), height (cm), and waist and hip circumference (cm) measurements were taken for all patients by the same investigator. The body mass index (BMI; kg/m^2) and waist-to-hip-ratio were also calculated.

Serum lipids and blood glucose

After an overnight fasting period, a blood sample was drawn from an antecubital vein to measure total cholesterol, triglycerides, and high-density lipoprotein cholesterol. Serum lipids were analyzed using a VITROS DT60 (Chemistry System Ortho-Clinical Diagnostics Inc., Rochester, NY, USA). Low-density lipoprotein cholesterol was calculated using the following equation: LDL = TC - HDL - (TRIG/2). Plasma glucose was determined using a Biosen C-line (EKF-diagnostic, Barleben/Magdeburg, Germany).

Intervention

MIAST sessions were held twice a week for 21 weeks at a local gym. Each exercise session lasted for 60 minutes and consisted of 10 minutes of warm-up, 20 minutes of aerobic training using a cycle ergometer, 20 minutes of strength training exercises, and 10 minutes of cool-down by stretching.

The target heart rate was set at the beginning of the training at 50-60% of the heart rate reserve (HRR) attained on the baseline exercise stress test, and it increased progressively up to 85% of the HRR during the intervention period. The Karvonen formula ([(HRmax - HRrest) × (0.50 to 0.85)] + HRrest) was used to calculate the target heart rates. The cycling periods were performed as interval-type aerobic training, and the intensity of the training varied according to the program. During the training sessions, a wireless computerized electrocardiograph (ECG) monitoring system (Wi-EKG T30, CorusFit Inc., Jyvaskyla, Finland) was used to monitor the participants' heart rates and functions [19].

After the 20 minutes of interval-type aerobic training, the participants performed strength training exercises. The program included leg press and functional exercises only for the large muscle groups of the lower body. Patients performed two to three sets of 12 repetitions, with an intensity of 50-70% for one repetition maximum (1 RM) on a horizontal bi-/unilateral leg press. Functional lower extremity exercises (squat/step-up-squat, step-up, and heel rise) were planned, with body weight and body weight adjusted with dumbbells used as resistance. In addition, abdominal exercises were performed with two sets of 10-20 repetitions for all movements. The exercise group members recorded their intervention exercise sessions in training diaries. The control group was asked to carry out their usual daily activities.

Lymphomonocyte gene expression sub-study

Seven men (mean age 59.3, SD 7.7) from the exercise group enhanced their peak oxygen uptake (VO_{2peak}) during the 21-week training period and were included in this sub-study.

Mononuclear cells were separated from the fasting venous blood samples taken before and after the exercise intervention using Becton Dickinson Vacutainer cell preparation tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) with sodium citrate in accordance with the manufacturer's guidelines. Total RNA from mononuclear cells was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA). The detailed methods of the RNA quality controls and amplification are described in [20]. Hybridizations and raw data readings from Illumina HumanWG-6 v3.0 Expression BeadChips (Illumina Inc., San Diego, CA, USA), containing probes for 48,803 transcripts, were performed by the Finnish DNA Microarray Center at the Turku Center for Biotechnology according to the Illumina BeadStation 500× manual (Revision C) using Illumina BeadArray Reader (Illumina Inc.) and BeadStudio v3 software (Illumina Inc.). After initial data analyses, the normalization and statistically computed fold change (FC) between the baseline and the twenty-first week of training for gene expression and the *p*-value of each gene were used

Table 1. Baseline characteristics of the patients

	Training group (n = 12)	Control group (n = 11)
Men/Women	11/1	9/2
Age (y)	58.6 ± 8.5	63.3 ± 6.1
Ejection Fraction (%)	60.7 ± 9.7	60.1 ± 9.3
Disease history		
PCI (n)	12	11
MI silent (n)	3	1
CABG (n)	3	3
Stroke (n)	1	0
Transient ischemic attack (n)	1	1
Arterial hypertension (n)	5	7
Type II diabetes (n)	3	2
Asthma (n)	1	3
Spinal stenosis (n)	1	1
Medications		
β-blockers (n)	11	9
Statin (n)	11	11
Nitrates (n)	2	2
Ca^{2+}-blockers (n)	2	2
ACE inhibitors (n)	1	4
AT-blockers (n)	1	4
Diuretics (n)	1	2
Aspirin (n)	11	10

Mean ± DS unless otherwise indicated; PCI = Percutaneus Coronary intervention; MI = Myocardial infarction; CABG = Coronary Artery Bypass Grafting; ACE = Angiotensin-Converting Enzyme inhibitiors; AT = Angiotensin receptor blockers.

for functional analyses using IPA analysis (Ingenuity Pathway Analyses, Qiagen, USA). Through IPA analysis, the functional areas of the leukocytes that were affected by effective exercise were identified. The gene expression data and the raw data sets are available in the GEO database (accession number GSE133405). MIAME guidelines were followed during array data generation, preprocessing, and analysis.

Statistical analysis

All analyses were performed on an intention-to-treat principle. The means and standard deviations (mean ± SD) of the parameters of both groups were used in the comparisons. The normality of the variables was analyzed using the Shapiro-Wilk test. The differences between the groups were tested using the Student's t-test for unpaired samples at the baseline. The absolute and relative changes between the groups after the 21-weeks of training were analyzed using analysis of variance with repeated measures (ANOVA). In addition to analyzing effects between groups and effects over time during the 21-week training period, contrasts between weeks 0 and 12, 12 and 21, as well as 0 and 21 were also analyzed. The software package SPSS statistics (SPSS Inc, Chicago, IL, USA) and a criterion of $P < 0.05$ were used.

The analyses of the gene expression array and normalization were conducted in R using the Bioconductor module and the Limma package for group comparisons (R-project, bioconductor, limma) (for details, see [20]). The raw data of each chip were quantile-normalized, and the data quality was assessed by calculating the Pearson correlation coefficients and clustering. For each gene, the FC, the t-test *p*-value, and false discovery rate adjusted *p*-value were calculated between the baseline and the twenty-first week of training. The sample pair effect was taken into account in all testing. For IPA analyses, the gene list was filtered to include genes with an FC greater than |1.10| and *p*-value smaller than 0.05. Fisher's exact test was used for *p*-value calculations of significance in all functional analyses and pathways, with *p*-values smaller than 0.01 considered significantly enriched. Pathway activation or inactivation was predicted with z-scores, with z-scores more than 2 considered activated and z-scores less than -2 considered inhibited.

Results

One patient from the control group dropped out of the study due to personal reasons prior to the measurements at 12 weeks. As all statistical analyses were performed based on the intention-to-treat principle, the baseline values for this patient were included as inputs in the analyses at 12 and 21 weeks. The baseline characteristics were comparable between the groups (**Tables 1** and **2**). Eleven patients in the training group and nine in the control group used β-blockers in accordance with the national coronary artery disease treatment guidelines. The training group completed on average 36 of 42 training sessions (87%; range

Table 2. The results of study variables at baseline, week 12 and week 21 in training group (Ex) and control group (Cont)

	Baseline (week 0)		Week 12		Week 21		0 vs. 12 vs. 21 wk#			Contrasts		
	Ex (n = 12) Mean (SD)	Cont (n = 11) Mean (SD)	Ex (n = 12) Mean (SD)	Cont (n = 11) Mean (SD)	Ex (n = 12) Mean (SD)	Cont (n = 11) Mean (SD)	Time x Group	Time	Group	0 vs. 12	12 vs. 21	0 vs. 21
Anthropometry												
Weight (kg)*	84.2 ± 13.9	83.2 ± 15.2	84.2 ± 14.1	83.3 ± 15.7	82.9 ± 14.1	82.9 ± 15.6	.284	.045	.918	.974	.168	.272
BMI	27.1 ± 3.0	27.9 ± 4.2	27.1 ± 3.0	27.9 ± 3.0	26.7 ± 2.9	27.8 ± 4.3	.317	.045	.578	.940	.181	.302
Waist circumference (cm)	97.4 ± 9.1	97.9 ± 13.6	97.2 ± 9.4	97.2 ± 9.4	95.9 ± 9.8	97.7 ± 13.6	.134	.100	.858	.829	.057	.177
Hip circumference (cm)	100.9 ± 5.9	101.3 ± 6.8	101.1 ± 6.3	101.1 ± 6.3	100.3 ± 6.2	101.0 ± 7.2	.170	.017	.863	.052	.085	.983
Waist-hip ratio	0.96 ± 0.05	0.96 ± 0.08	0.96 ± 0.05	0.96 ± 0.08	0.96 ± 0.06	0.96 ± 0.08	.088	.718	.990	.160	.246	.065
Serum												
Blood glucose (mmol/l)	5.8 ± 1.1	5.4 ± 0.9	5.7 ± 1.2	5.2 ± 0.8	5.3 ± 1.0	5.2 ± 0.9	.195	.016	.358	.330	.050	.439
Total cholesterol (mmol/l)	4.1 ± 1.0	3.9 ± 0.8	4.1 ± 1.0	3.8 ± 0.6	3.5 ± 0.5	3.9 ± 0.7	.032	.086	.939	.566	.019	.034
HDL cholesterol (mmol/l)	1.1 ± 0.3	1.2 ± 0.5	1.2 ± 0.3	1.3 ± 0.4	1.1 ± 0.3	1.3 ± 0.4	.059	.183	.411	.201	.005	.352
LDL cholesterol (mmol/l)	2.0 ± 1.0	2.0 ± 0.5	2.3 ± 0.9	1.9 ± 0.6	1.8 ± 0.4	1.9 ± 0.6	.185	.310	.626	.215	.038	.721
Triglycerides (mmol/l)*	1.9 ± 2.2	1.4 ± 0.7	1.2 ± 0.4	1.3 0.7	1.2 ± 0.6	1.4 ± 0.7	.271	.205	.850	.322	.203	.591
Muscle strength and CSA												
Knee extension (N)	545.4 ± 179.2	474.3 ± 159.5	598.4 ± 180.9	502.5 ± 139.9	645.2 ± 189.2	506.5 ± 143.7	.022	.000	.157	.311	.019	.018
CSA of QF (cm^2)	68 ± 15	63 ± 15	x	x	70 ± 17	61 ± 14	.012	.972	.291			
LCSA of QF (cm^2)	65 ± 15	59 ± 16	x	x	67 ± 16	57 ± 15	.003	.714	.231			
Spiroergometry												
Heart rate (rest; beats/min)	53.9 ± 3.9	51.7 ± 6.7	54.4. ± 4.4	56.5 ± 8.8	54.4 ± 4.5	54.4 ± 6.8	.101	.033	.976	.008	.324	.328
Systolic BP (rest; mm Hg)	129.7 ± 12.5	134.6 ± 18.2	134.2 ± 12.3	142.9 ± 24.0	140.2 ± 17.6	138.2 ± 17.1	.094	.004	.594	.429	.068	.145
Diastolic BP (rest; mm Hg)	77.5 ± 4.8	78.4 ± 8.5	78.1 ± 7.1	80.7 ± 8.2	77.3 ± 6.8	80.9 ± 7.5	.528	.441	.387	.446	.722	.267
Heart rate (max; beats/min)	148.7 ± 15.9	142.3 ± 19.2	147.7 ± 19.1	144.6 ± 16.9	143.8 ± 15.7	139.8 ± 14.0	.739	.116	.502	.453	.850	.561
Systolic BP (max; mm Hg)	217.5 ± 19.2	215.8 ± 21.5	211.3 ± 21.5	214.9 ± 24.7	217.2 ± 23.9	212.6 ± 25.8	.354	.468	.920	.403	.128	.612
Diastolic BP (max; mm Hg)*	87.2 ± 10.1	93.1 ± 9.4	84.2 ± 12.4	90.9 ± 10.4	86.7 ± 10.7	91.8 ± 10.8	.888	.282	.158	.814	.658	.782
Exercise time (max; s)	719.0 ± 199.1	624.9 ± 159.5	799.3 ± 255.6	624.0 ± 179.3	779.3 ± 227.9	618.1 ± 171.7	.069	.095	.102	.033	.729	.053
Work load (max; W)	174.6 ± 41.4	151.2 ± 41.1	191.7 ± 53.3	154.8 ± 37.9	187.1 ± 47.9	153.5 ± 35.9	.240	.043	.089	.117	.703	.213
Peak VO_2 (max; ml/kg/min)	25.8 ± 5.3	23.2 ± 4.9	27.6 ± 6.5	22.4 ± 4.9	27.5 ± 5.0	23.5 ± 4.8	.063	.179	.079	.025	.293	.173

= General Linear Modle for Repeated Measures; * = Non-normally distributed variables; Time x Group = all 3 time points and both groups; Contrasts = 2 timepoints and both groups; x = Not measured; Ex = Exercise group; Cont = Control group; BMI = Body Mass Index; HDL = High Density Lipoprotein; LDL = Low Dnesity Lipoprotein; CSA = Cross-sectional area; LCSA = Lean Cross-sectional area; QF = Quadriceps Femoris; BP = Blood Pressure; VO_2 = Maximal oxygen uptake.

60-100%). The exercise group did not report any increasing symptoms. Wireless ECG monitoring during the training sessions did not reveal changes that would interrupted training.

Exercise stress test

The 21-week training period led to improvements in VO_{2peak} (interaction P = 0.063) (**Table 2**) and in the duration of the exercise test (borderline effect P = 0.069) (**Table 2**) in the training group compared to the control group. Contrasts between the two time points showed that during the first 12 weeks of the training, VO_{2peak} (6.9 ± 11.1% vs. -3.3 ± 9.2%; P = 0.025) and exercise test duration (11.2 ± 14.8% vs. 0.4 ± 5.9%; P = 0.033) improved significantly more in the training group than in the control group. No other significant changes were observed between the groups in the maximal spiroergometry results. The absolute and relative changes in the maximal spiroergometry results did not correlate with the absolute and relative changes of muscle force and CSA or with the values for serum glucose and lipids.

One of the physicians interrupted the maximal spiroergometry for three patients in the training group due to ST-segment depression. The ST-segment depression was non-symptomatic, without any other symptoms of ischemia such as chest pain. When these three patients were removed from the analyses, the VO_{2peak} (12.2 ± 11.7% vs. 1.9 ± 6.1%; P = 0.015), the test duration (16.1 ± 9.3% vs. -0.2 ± 5.5%; P < 0.001), and the work load (13.4 ± 7.5% vs. 2.8 ± 12.0%; P = 0.005) increased significantly in the training group compared to the control group during the 21-week training period.

Maximal isometric muscle force and muscle composition

The maximal isometric force of the knee extensors increased significantly in the training group compared to the control group between the baseline and week 21 (19.9 ± 15.6% vs. 9.5 ± 9.6%; P = 0.022) (**Table 2**).

After the training period, the observed 2.2 ± 5.5% increase in the CSA of QF in the training group differed significantly from the observed decrease of 2.6 ± 3.9% in the control group (P = 0.012). The changes in LCSA also differed between the groups (P = 0.003). Specifically, LCSA increased by 2.4 ± 4.7% (from 65 ± 15 to 67 ± 16 cm^2) in the training group and decreased by 3.4 ± 4.2% (from 59 ± 16 to 57 ± 15 cm^2) in the control group after the 21-week training period.

Serum lipids and plasma glucose

The change in the serum total cholesterol concentration differed significantly between the groups after the completion of the intervention period (training group -12 ± 3%; from 4.1 ± 1.0 to 3.5 ± 0.5 mmol/l vs. control group 1.0 ± 4.2%; from 3.9 ± 0.8 to 3.9 ± 0.7 mmol/l) (P = 0.034). Contrasts between the two time points showed that significant changes in the blood glucose and cholesterol values occurred during the last half of the training period (**Table 2**). However, the changes between the baseline and the 21-week follow-up measurements were not significant. No other significant changes were observed between the groups in the serum lipid and plasma glucose concentrations (**Table 2**).

Lymphomonocyte gene expression sub-study

In addition to enhancing their peak oxygen uptake (P = 0.009), the sub-study subjects improved their exercise time (P = 0.001) and maximum workload (P = 0.001) in the spiroergometry test during the 21-week training period. The lean mass of the quadriceps femoris (P = 0.038) and the knee extension force (P = 0.015) improved as well. No statistically significant differences were seen in the anthropometric measurements between the baseline and the twenty-first week of training. Total cholesterol levels decreased even though triglycerides, LDL, and HDL cholesterol did not show statistically significant differences between the baseline and the twenty-first week of training. Blood glucose and high sensitive C-reactive protein (CRP) remained unchanged.

With our filtering criteria (P < 0.05; |FC| > 1.1), we identified 2,038 genes being regulated (960 down and 1078 up) in lymphomonocytes in parallel with increased maximal oxygen uptake during the 21 weeks of training. An enrichment analysis of the genes on canonical pathways with IPA brought up two functional areas: oxygen metabolism and immune response. The oxidative phosphorylation pathway and glutathione redox reactions I enzymes we-

Table 3. Canonical pathways regulated after the 21-week training compared to the baseline in patients with stable CAD

Ingenuity Canonical Pathways	-log (*p*-value)	Ratio	z-score
Mitochondrial Dysfunction	5,47	0,24	NA
Oxidative Phosphorylation	**3,62**	**0,24**	**3,27**
Tec Kinase Signaling	**3,46**	**0,20**	**-2,04**
Cell Cycle: G2/M DNA Damage Checkpoint Regulation	3,39	0,29	0,00
Inflammasome pathway	3,21	0,40	-0,71
T Helper Cell Differentiation	3,08	0,25	NA
Glutathione Redox Reactions I	**2,89**	**0,36**	**2,83**
Protein Kinase A Signaling	2,82	0,16	-0,75
Prostate Cancer Signaling	2,69	0,21	NA
Lipid Antigen Presentation by CD1	2,62	0,37	NA
ERK5 Signaling	2,62	0,23	-0,26
p38 MAPK Signaling	2,59	0,20	-0,66
Acute Myeloid Leukemia Signaling	2,54	0,21	-0,50
Death Receptor Signaling	2,48	0,21	-1,15
Huntington's Disease Signaling	2,48	0,17	0,47
ILK Signaling	2,44	0,17	0,35
Epithelial Adherens Junction Signaling	2,36	0,18	NA
Aryl Hydrocarbon Receptor Signaling	2,36	0,18	-0,26
PI3K/AKT Signaling	2,35	0,19	-0,23
Integrin Signaling	2,33	0,17	1,22
Cdc42 Signaling	2,31	0,19	0,78
FLT3 Signaling in Hematopoietic Progenitor Cells	**2,24**	**0,21**	**-2,18**
IL-7 Signaling Pathway	**2,24**	**0,21**	**-2,67**
Dendritic Cell Maturation	2,18	0,17	-1,13
Phospholipase C Signaling	2,12	0,16	0,00
Th1 and Th2 Activation Pathway	2,09	0,17	NA
IL-8 Signaling	2,08	0,16	0,93
Ephrin Receptor Signaling	2,06	0,17	-0,41

Ingenuity Pathway-analysis was conducted with gene list filtered with FC > |1.10| and P < 0.05 and pathways with P < 0.01 were considered significantly regulated. Pathways with z-score > |2| are highlighted with bold font.

re up-regulated after training (z-score > 2, **Table 3**). Interestingly, gene expression in the mitochondrial respiratory complex proteins were significantly up-regulated, with the exception of complex II. The expression of genes in complex II, especially *SDHA* and *SDHC*, was down-regulated. Several immune response-related pathways, such as the Tec kinase signaling pathway, the inflammasome pathway, and the T helper cell differentiation pathway, were enriched significantly within the genes regulated during training (**Table 3**). The expression of these pathways tended to be downregulated (z-score < -2), giving the highest confidence levels for the Tec kinase signaling pathway, the FLT3 signaling pathway, and the IL-7 signaling pathway.

Discussion

We examined the effectiveness of a 21-week MIAST program on aerobic capacity, muscle strength and composition and cardiovascular risk factors in patients with stable CAD who had not yet suffered MI. The results indicate that the program, which included interval-type aerobic training, significantly increased VO_{2peak} and exercise time in the aerobic test and improved muscle strength and mass in the knee extensors. The changes in VO_{2peak} and exercise time were observed after 12 weeks of regular, supervised exercise training. Serum lipids and blood glucose tended to decrease during the second half of the 21-week training period. The results suggest that the MIAST program was

safe, as no complications or injuries occurred during the training sessions. In addition, results show that an increase in oxygen metabolism and a decrease in immune response gene expression in the subjects' lymphomonocytes enhanced their VO_{2peak} during the intervention.

The current exercise recommendations for patients with CAD are mainly directed to those who have already suffered MI [6]. However, effective regular exercise is especially important for patients with CAD who have not yet suffered MI, as it could prevent the progression of CAD and improve their overall physical fitness. Traditionally, steady-state training has been preferred to interval training because it was thought to be safer. However, in our study as well as in some previous studies [8-11, 13], participants performed interval-type aerobic training, that entailed a higher intensity of exercise than that used in stable aerobic training. Despite this high intensity, the training was shown to be safe, as our study, in line with previous studies, did not report any adverse effects.

The MIAST program was carried out twice a week in our study, and the total amount of time undertaken by both training groups was only one hour per week, which is clearly less than current exercise recommendations suggest [5]. However, the MIAST program still led to a 6.9% increase in VO_{2peak} and an 11.2% increase in exercise time after 12 weeks of training. During the last nine weeks of training, VO_{2peak} did not improve despite the fact that the program was progressive. Most likely the frequency, duration, or intensity of the aerobic exercise remained too low after the first 12 weeks, and thus, the interval-type aerobic training performed twice a week may not have been sufficient to improve VO_{2peak} and achieve continued improvements after the 12 weeks of training. Volaklis et al. [21] compared two CAD and MI patients groups: a group that combined circuit weight training and aerobic training and control group that did not undergo any training. In their study, the patients in the training group completed four training sessions per week for eight months, with the training intensity increasing from 60-75% to 70-85% of the maximal heart rate during the training period. Their results showed that the training led to a 15% increase in VO_{2peak} and a 14% increase in exercise time. In addition, a meta-analysis [22] found that VO_{2max} increased by 14% in training groups, compared with a 2% increase in control groups in patients with coronary artery heart diseases. Our results are well in line with these results when our 21-week study period and low training frequency are taken into account.

It is also important to consider that improvements were observed after 12 weeks even though all but two of the participants used β-blockers, a medication that sets physiological limits on increases in heart rate and aerobic capacity. This finding is supported by earlier studies in which most participants used β-blockers and yet improved oxygen uptake significantly [8-10]. In spite of β-blocker use, study participants have been found to easily and safely exercise at changing aerobic exercise intensities and experience positive changes in oxygen uptake. Therefore, we suggest that interval-type aerobic training is suitable for patients with stable CAD, bearing in mind that exercise prescriptions should be planned carefully for these patients to ensure the safety and effectiveness of the interventions.

In the present study, strength training was performed after aerobic training, and for safety reasons only large lower body muscle groups were loaded. Muscle strength in the knee extensors was found to increase by 20%, and the muscle CSA and LCSA of the QF also increased, indicating that the training affected both muscle strength and composition. Other studies have reported similar results for muscle strength (28%) after eight months of combined training [21] and for body composition after one year of aerobic and combined training in patients with CAD [23]. These results indicate that it would be possible to achieve positive changes in muscle strength and muscle/body composition in CAD patients after 21 weeks of regular resistance training.

A tendency towards improvements in cardiovascular risk factors was observed in our study after the 21 weeks of training. A previous review found that although aerobic training reduced concentrations of serum risk factors [4], changes after strength training were not unclear [24, 25]. Eight weeks of strength training was not enough to produce significant lipid profile changes in older women [24], while bo-

th 10-week and 16-week aerobic and strength training periods had positive effects on plasma lipoprotein levels in healthy, active, older women [25, 26]. Our results suggest that the MIAST program led to significant changes in the lipid profile in CAD patients using statins possibly due to exercise-induced changes in muscle tissue metabolism. However, as these results show, the effects of strength training on blood lipid profile are controversial, and more studies are needed.

Despite a relatively small sample size, we observed improvements in physical fitness among the CAD patients, who were generally in good physical condition due to the fact that their diagnosis had been made several months previously. However, the results indicate that these patients may benefit significantly from regular and intensive exercise. In our study all physicians, who supervised the exercise stress tests, were blinded for the study groups, but unfortunately the common criteria for interruption of the exercise stress test were lacking, and this may have affected VO_{2peak} results.

The enhanced oxygen up-take among CAD patients resulted to modulation of gene expression profile in lymphomonocytes. Our inclusion criteria for this sub-study was enhanced peak oxygen uptake, which was also reflected in the increased expression of the oxidative phosphorylation gene-set in lymphomonocytes. Our result is supported by the enhanced gene expression of the oxidative phosphorylation genes in the whole blood samples taken in a group of subjects who responded highly to the exercise during the 12-week training period [27]. These results suggest effective exercise training to increase mitochondrial function, which in lymphomonocytes is associated with better health [17, 28]. Physical exercise and increased mitochondrial function are also known to increase the production of reactive oxygen species (ROS). The activation of genes related to glutathione redox reactions in lymphomonocytes suggest an increase in reducing power which protects cells against oxidative stress [29]. In addition, mitochondrial complex II is one of the mitochondrial ROS producers, and the observed decrease in the expression of complex II genes may also be part of oxidative stress management. This is supported by findings that the mitochondrial complex II subunits SHDA and SHDC, whose gene expression was diminished, are the subunits that take part in ROS production [30].

The Tec kinase, FLT3, and IL-7 signaling pathways are all related to the proliferation, differentiation, activation, and apoptosis of lymphocytes. Down-regulation of these pathways suggests increased apoptosis, decreased proliferation, differentiation, and activation of lymphomonocytes. This observation is supported by a recent voluntary running study of mice, which showed that exercise reduced inflammatory cell proliferation, mobilization, and differentiation in several steps on the way to mature leukocytes [31]. Cardiometabolic diseases, as well as ageing, are known to be associated with chronic low-grade inflammation that can be attenuated with physical exercise [32]. Observed down-regulation of inflammatory pathways may be a sign of this, or it may be associated with adaptations of innate immunity after training [33]. Further study is needed of the changes in the distribution and gene expression of immune cell populations to understand immune system changes induced by exercise training. Because ROS are also important in immune system regulation [34], this sub-study suggests that the importance of redox balance in the health-promoting effects of exercise training among CAD patients warrants further study.

In conclusion, the MIAST program, which was carried out twice a week for 21 weeks, was shown to improve physical fitness in patients with stable CAD who had not yet suffered acute MI. Improvements in VO_{2peak} were observed after only 12 weeks of training. During the last nine weeks of training, muscle strength improved but further improvements in VO_{2peak} or cardiovascular risk factors would have most likely required modifications to the training program. However, enhanced oxygen up-take among the CAD patients was associated with changes in gene expression in leukomonocytes, which suggests that increases in mitochondrial function and decreases in inflammation may be health-enhancing mechanisms. In addition, the MIAST program was safe and suitable for patients with stable CAD using β-blocker medication, and it did not cause any injuries or adverse effects. Thus, this type pf exer-

cise program, including interval-type aerobic training, can be recommended for inclusion in rehabilitation programs for stable CAD patients with no history of MI.

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Disclosure of conflict of interest

None.

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