High-intensity Interval Training after Percutaneous Coronary Intervention

A randomized controlled study evaluating the effect of exercise training on late luminal loss with relationship to inflammation and endothelial function

Peter Scott Munk

Dissertation for the degree of philosophiae doctor (PhD)
University of Bergen, Norway
2010

UNIVERSITY OF BERGEN

High-intensity Interval Training after Percutaneous Coronary Intervention

A randomized controlled study evaluating the effect of exercise training on late luminal loss with relationship to inflammation and endothelial function

Peter Scott Munk

Dissertation for the degree of philosophiae doctor (PhD)

University of Bergen

2010

Dissertation date: 22nd October 2010

Institute of Medicine, Faculty of Medicine and Dentistry, University of Bergen, Norway

Stavanger University Hospital, Stavanger, Norway

Scientific environment

Professor Dr. med. Alf Inge Larsen

Institute of Medicine, Faculty of Medicine and Dentistry, University of Bergen, Bergen and

Department of Cardiology, Stavanger University Hospital, Stavanger

Contents

To Ane Cecilie, Christopher, Julian and Victoria

Acknowledgements

The present work is based on studies performed at Stavanger University Hospital and Stavanger Health Research in the period January 2006 to September 2008. A research fellowship from the Norwegian Health Association (Nasjonalforeningen for Folkehelsen) was the primary source of financial support. A short term scholarship from the "Kompetansesenter for klinisk forskning", Haukeland University Hospital, Bergen and grants from the local research fund of Helse Stavanger were important contributions.

My interest in experiments and research originates from my childhood and was stimulated by my parents. My father graduated with a Philosophiae Doctor degree at the University of Tübingen, followed by a post-doc scholarship at the University of California, San Diego, USA. During medical school at the University of Freiburg I was introduced to experimental research by Professor Dr. Gerd Hasenfuss at the department of cardiology, resulting in a "German doctorate" in 1997.

In 2005 I was a cardiology fellow and was looking for a supervisor to start a scientific project. My choice fell on Professor Alf Inge Larsen, who coincidentally became my supervisor at that time. During our first meeting I informed him about my interest in research and asked for a scientific project that might result in a PhD. He suggested an evaluation of exercise training on inflammation and coronary artery disease progression as an expansion and continuation of his own thesis. Our first meeting was the beginning of a fruitful collaboration, which resulted in the present work.

Starting with our hypothesis, that a "modern" high-intensity interval training program would result in less restenosis and inflammation after stenting of coronary arteries, it took only a few weeks until the protocol was written and approved by the local ethic committee. The infrastructure for the training was set up, and the physical therapists Marit Haugland and Anne Kristiansen were interested in training our patients using the "Trondheim" high-intensity interval training model. However, it took a great amount of effort and patience to get financial support for our project. At this point I am very grateful to Leik Woie, the head of the cardiology department at that time, for his generous support. We ran a scientific project sponsored by the pharmaceutical industry together and invested the profit and our payment in my project.

During the first two years my grants were declined and the study had to be run on top of my clinical work prolonging my working hours to the evenings and weekends.

It was a big relief and support when finally Carina Alm, the head of cardiovascular research at the Norwegian Health Organisation (Nasjonalforeningen for Folkehelsen) offered me a scholarship for three years.
I am very grateful to my supervisor Alf Inge Larsen. Without his positive and open attitude, continuous encouragement and enthusiasm during these years this work would have been impossible. He responded always within short time giving my questions and queries highest priority.

Further, I am grateful to Marit Kristiansen and Anne Haugland for their expert assistance in planning, organizing and running the training program. Their continuous motivation of patients participating in the training sessions made a major contribution to the study.
I also want to express my gratitude to my co-authors, Eva Staal, Noreen Butt and Kjetil Isaksen and Unni Mathilde Breland for their contributions. A special thanks to my colleague and co-author Øyvind Skadberg, who was always positive and encouraging in terms of collaboration.
A special thanks also to Torbjørn Aarsland, the chief at Stavanger Health Research, for introducing me to ergospirometry. His multiple skills and experience were of great value for my work. Further, I want to thank Anne Gro Larsen, Jorunn Nilsen, Solfrid Moen and Helles Svanes, it was a pleasure working with You.
During the process of the two papers dealing with inflammation I had the pleasure of working with Pål Aukrust and Thor Ueland, whose knowledge and experience in this field was very much appreciated.
The assist of my colleagues, Ståle Barvik, Vernon Bonarjee, Tor Melberg and Dennis Nilsen, during invasive procedures was very much appreciated.
The staff and nurses at the department of cardiology were always positive and helpful in dealing with study patients, where research often interferes with daily routines, thank You.
Finally, I also want to thank Sverre Uhlving, head of the Department of Medicine and Cord A. Manhenke, head of the Department of cardiology, Stavanger University Hospital for their support of my research.
Last but not least I want to thank my family. Scientific work has always been considered as highly respectable and desirable in my parents' house. The freedom and flexibility of being a research fellow has been an advantage for family life, but working late hours at home means loss of spare time. Thank You, Ane Cecilie for continuous support, understanding and love. Thank you, Christopher Robin, Julian and Victoria for supporting me.

Abbreviations

PCI: Percutaneous coronary intervention

CABG: Coronary artery bypass grafting

CAD: Coronary artery disease

MI: Myocardial infarction

BMS: Bare metal stent

DES: Drug eluting stent

CRP: C-reactive protein

PTX3: Pentraxin-3

IL-6/8/10: Interleukin-6/8/10

TNFα: Tumor necrosis factor α

vWF: von Willebrand factor

VCAM-1: Vascular cell adhesion molecule-1

CD40L: CD40 ligand

MCP-1: Monocyte chemoattractant protein

RANTES: *r*egulated on *a*ctivation, *n*ormal *T* cell *e*xpressed and *s*ecreted

CCL19: Chemokine (C-C motif) ligand 19

CCL21: Chemokine (C-C motif) ligand 21

CXCL16: Chemokine (C-X-C motif) ligand 16

EDTA: ethylenediaminetetraacetic acid

EIA: Enzyme immunoassay

MLD: Minimal lumen diameter

LLL: Late luminal loss

FMD: Flow-mediated vasodilation

HRV: Heart rate variability

SDNN: Standard deviation of all NN intervals in the entire 24 hour registration

SDANN: Standard deviation of the average normal RR intervals for 288 5-minute segments of a 24 hour ECG recording

TINN: Triangular interpolation of NN interval histogram

RMSSD: Root mean square of differences between successive NN intervals

Abstract

Introduction

In spite of consistent scientific documentation of the beneficial effects of exercise training programs in coronary artery disease (CAD), exercise training is a remarkably underutilized (1) and probably also undervalued treatment option. CAD is an inflammatory disorder linking inflammation in the vessel wall to endothelial dysfunction and progression of disease (2). The aim of this study was to explore the effects of a high-intensity interval training program on the late luminal loss and the associated risk of restenosis, plasma levels of inflammatory markers, endothelial function and the cardiac autonomic nervous system.

Further, percutaneous coronary intervention (PCI) can be regarded as a model for mechanical induced plaque rupture. An additionally objective was therefore the evaluation of the inflammatory response to PCI reflected by plasma levels of numerous inflammatory mediators.

Methods

Subjects

Forty consecutive patients were included after successful PCI with stent implantation in one or several native coronary arteries because of angina pectoris. Patients were randomly assigned to an organized training program for 6 months or to usual care.

Exercise Training

A high-intensity interval group training for 60 minutes three times a week was used, starting 11±4 days after PCI. Individual pulse watches allowed the patients to monitor their heart rate and exercise intensity during training.

Quantitative coronary angiography

At 6 months follow-up, coronary reangiography with repetition of identical angiographic projections of the lesion was performed. A validated computer-assisted edge-detection algorithm was used to measure late luminal loss defined as the minimal lumen diameter immediately after the procedure minus the minimal lumen diameter at 6 months follow-up.

Exercise Testing

Symptom-limited ergospirometry was performed on an upright, electrically braked cycle ergometer using a 20 Watt/minute ramp protocol. The patients were asked to exercise to

exhaustion. Gas exchange data were collected continuously with an automated breath-by-breath system.

Endothelial Function

Endothelium-dependent and endothelium-independent dilation of the brachial artery was measured using a 12-MHz ultrasound Doppler probe according to current guidelines (3).

Inflammatory markers

High-sensitivity CRP concentrations were measured by a particle-enhanced immunoturbidimetric method with the use of Roche Modular P automated clinical chemistry analysers (Roche Diagnostics). Plasma concentrations of interleukin-(IL)-6, IL-10, pentraxin 3 (PTX3), tumor necrosis factor (TNF)α, vascular cell adhesion molecule (VCAM)-1, E-selectin, P-selectin, monocyte chemoattractant protein (MCP)-1/CCL2, *r*egulated on *a*ctivation, *n*ormal *T* cell *e*xpressed and *s*ecreted (RANTES/CCL5), CCL19, CCL21, IL-8/CXCL8 and CXCL16 were quantified by enzyme immunoassays (EIAs) obtained from R&D Systems (Minneapolis, MN). Plasma concentrations of von Willebrand factor (vWF) were analyzed by EIA using antibodies from DakoCytomation (Glostrup, Denmark). Plasma concentrations of CD40 ligand (CD40L) were analyzed by EIA provided by Bender MedSystems GmbH (Vienna, Austria).

Heart Rate Variability

Heart rate variability (HRV) measures were obtained from a 3-channel portable Holter (Schiller MT-200, Switzerland) worn for 24 hours. The MT-210 Analysis Software Version 1.0.0 (Schiller) was used to analyse time and frequency domain measures of HRV.

Results

At six months in-stent restenosis, measured as in-segment late luminal loss of the stented coronary area, was significant smaller in the training group compared to the control group. This effect was associated with a significant increased peak oxygen uptake and improved brachial artery reactivity in the training group only.

In the training group all time domain indices and the frequency domain indices, total power and ultralow frequency of HRV, increased significantly during the training period, while mean heart rate decreased significantly. In the control group only one time domain measure index increased significantly.

Plasma levels of CRP and PTX3 showed a significantly early increase after PCI peaking at 3 days and 3 hours, respectively. VCAM-1 increased significantly with a peak at 3 days, while

E-selectin showed a significant gradual decrease. Markers of platelet mediated inflammation showed increasing (CD40 ligand) and decreasing (P-selectin) levels after PCI. While plasma levels of MCP, CCL21 and CXCL16 increased rapidly in response to PCI, IL-8, CCL19 and RANTES decreased.

At 6 months, levels of the inflammatory markers CRP, IL-6 and IL-8 were significantly reduced and levels of the anti-inflammatory cytokine IL-10 were significantly increased in the training group only. The changes of CRP and IL-6 from baseline to 6 months were significantly correlated to late luminal loss in the stented segment at 6 months. In contrast to these anti-inflammatory effects, training had no effect on markers of platelet-mediated inflammation, and the effect of training on markers on endothelial cell activation were rather complex showing attenuating (vWF) and enhancing (E-selectin and VCAM-1) effects..

Conclusion

Regular high-intensity interval exercise training over 6 months resulted in a significant reduction in late luminal loss in the stented coronary segment after PCI for angina pectoris. This effect was associated with increased aerobic capacity, improved endothelium function and improved heart rate variability. PCI induced a complex response of plasma levels of inflammatory markers and cytokines during the first week post-PCI.

Regular exercise training in non-acute stable angina patients following PCI may attenuate some, but not all inflammatory pathways, potentially contributing to the beneficial effects of exercise training on restenosis.

List of papers

Paper I:

Munk PS, Staal EM, Butt N, Isaksen K, Larsen AI.

High-Intensity Interval Training May Reduce in Stent Restenosis Following Percutaneous Coronary Intervention with Stent Implantation. A randomized controlled study evaluating the relationship to endothelial function and inflammation.

Am Heart J 2009;158:734-41

Paper II:

Munk PS, Butt N, Larsen AI.

High-Intensity Interval Exercise Training Improves Heart Rate Variability in Patients following Percutaneous Coronary Intervention for Angina Pectoris.

Int J Cardiol 2009. Dec 3. [Epub ahead of print].

Paper III:

Munk PS, Breland UM, Aukrust P, Skadberg Ø, Ueland T, Larsen AI.

Inflammatory Response to Percutaneous Coronary Intervention in Stable Coronary Artery Disease.

J Thromb Thrombolysis. 2010 Apr 7. [Epub ahead of print]

Paper IV:

Munk PS, Breland UM , Aukrust P, Ueland T, Kvaløy JT, Larsen AI.

Exercise training reduces systemic inflammation in patients with stable coronary heart disease treated with percutaneous coronary intervention. Relationship to reduction of restenosis.

In revision.

1. Introduction

Despite the improvements of treatment strategies for hypercholesterolemia and hypertension, CAD remains the leading cause of death globally (4). These facts force us to continue research on cardiovascular disease and consider new strategies for prevention, treatment and rehabilitation of this prevalent disease.

1.1 Coronary artery disease and exercise

CAD is an inflammatory disease with accumulation of lipids in atheromatous plaques within the walls of the coronary arteries. Many of the independent risk factors for CAD like hypertension, dyslipidemia, diabetes and smoking are related to a sedentary lifestyle. Overweight has reached epidemic proportions also in Norway, where about 20% of the Norwegian adult population is considered obese (5).

Regular exercise training as a treatment modality has the ability to improve risk factor profile as training has been shown to improve glygemic control, lipid profile, blood pressure and body weight in different patient populations (6-8).

Current guidelines recommend all patients with CAD to exercise 3-5 days a week at 50-80% of exercise capacity for 20-60 minutes (9). Although the beneficial effects of exercise training in primary and secondary prevention of cardiovascular disease are well documented (10, 11), exercise training as a treatment modality is currently remarkably underutilized (1).

Recently, high-intensity aerobic interval training has been shown to be superior to moderate continuous exercise training in improving exercise capacity and endothelial function in patients with CAD, ischemic cardiomyopathy and metabolic syndrome (12-14).

1.2 Restenosis

PCI with stent implantation is the therapy of choice in patients with symptomatic CAD related to significant stenosis, not eligible to coronary artery bypass grafting (CABG). Although PCI and CABG may be comparable treatment strategies, restenosis remains the Achilles' heel of PCI.

Late loss in lumen diameter is monotonically related to the risk of restenosis in published stent trials and is a generalizable and powerful angiographic end point in stent trials (15). In stented arteries late luminal loss correlates primarily with intimal hyperplasia (16). Neointima is composed of proliferating smooth muscle cells and excessive production of extracellular

matrix (17). The formation of neointimal hyperplasia after PCI has been linked to persistent low local shear stress (18), to the inflammatory response to the vessel injury with increased levels of CRP (19) and to genetic factors involving regulation of smooth muscle cell and leukocyte proliferation in vascular disease (20).

The use of drug eluting stents (DES) has markedly reduced the restenosis rate (21), but efficiency and safety issues with regard to late and very late stent thrombosis are still under investigation in large randomized controlled trials.

1.3 Inflammation

Inflammation plays a key role in atherosclerosis (2). Immune cells accumulate early in atherosclerotic lesions and their effector molecules accelerate progression of the lesions (2). CAD is an inflammatory disorder, in which immune mechanisms interact with metabolic risk factors to initiate, propagate and activate lesions in the arterial tree (2).

Atheromata are asymmetric focal thickening of the intima consisting of lipids, connective tissue, debris and different cells as vascular endothelial cells, smooth muscle cells and blood-borne inflammatory cells like macrophages and T-cells (2, 22).

In the centre of an atheroma, foam cells and extracellular lipid droplets form a core region, which is surrounded by a cap of smooth-muscle cells and a collagen-rich matrix. T cells, macrophages and mast cells infiltrate the lesion and are particularly abundant in the shoulder region, where the atheroma grows (23, 24). Many of these immune cells show signs of activation and produce inflammatory cytokines (24), which may play an important pathophysiologic role for the development of the atheroma. The activation of a plaque with rupture of the cap can cause formation of a thrombus on the luminal surface of the plaque, resulting in an acute cardiovascular event.

In the current trial we studied the inflammatory response to PCI, which cause endothelial damage and barotrauma to the vessel wall. There are data indicating that this inflammatory response is of clinical importance, as it might be associated with the development of restenosis and increased risk for future coronary events (19, 25).

Further, the PCI procedure can be regarded as a model for mechanical induced plaque rupture. Thus, studies on the inflammatory reaction during PCI could give information on immune-mediated mechanisms during plaque destabilization.

The inflammatory response during PCI includes a complex interaction between endothelial cells, platelets and leukocytes, and involves a multitude of inflammatory mediators in a multifaceted network. Most studies on inflammation during PCI have focused on relatively

few mediators, primarily representing downstream markers of inflammation (e.g., CRP) and established upstream inflammatory mediators (e.g., IL-6, IL-1 and TNFα) (26, 27). We analyzed besides "classical" markers of inflammation, markers of endothelial cell activation, markers of platelet derived inflammation as well as chemokines that attract leukocytes to inflamed tissue.

Additionally, we were interested in the "anti-inflammatory" effect of regular exercise training as exercise training may lower the risk for CAD by attenuating inflammation and improving endothelial function.

1.4 Cardiac autonomic function

The autonomic nervous system is the part of the peripheral nervous system that controls key functions of visceral organs. It is classically divided into the parasympathetic nervous system and sympathetic nervous system.

The part of the autonomous nerve system affecting the heart comes from sympathetic ganglia running adjacent to the upper thoracic spinal column and the adrenal medulla. The parasympathetic innervation of the heart is mediated by dedicated cardiac branches of the vagus nerve and thoracic spinal accessory nerves (28).

Heart rate variability (HRV) measurements provide a non-invasive method to assess cardiac autonomic function. HRV increases with higher parasympathetic tone and decreases with sympathetic stimulation. Thus, higher HRV implies augmentation of parasympathetic tone, which might be protective against cardiac morbidity and mortality (29).

CAD is associated with cardiac autonomic imbalance reflected by low HRV. Low HRV is a negative prognostic factor for mortality and the risk for cardiac events in the general population (29). Data on the prognostic value of HRV in CAD without myocardial infarction (MI) or heart failure are limited. In healthy subjects exercise training results in a decrease of resting heart rate, believed to be caused by an increase in the parasympathetic tone (30). A meta-analysis showed that exercise training leads to improvements in HRV in patients with CAD (31). However, none of the randomized controlled trials published so far, have evaluated the effect of a high-intensity interval training program following revascularization with PCI and stent implantation in patients with CAD free of MI or heart failure.

Further, little is known about the mechanisms of the association between exercise training, autonomic imbalance and clinical outcome.

Therefore, we assessed the effect of a high-intensity interval training program on different day- and night- time measures of HRV. Further, we were interested in potential associations to changes in exercise capacity, endothelial function and inflammation.

"If we can selectively modify the harmful components of inflammation in the arteries and leave the protective aspects intact, we may create new avenues for the diagnosis and management of disease in the 50 percent of patients with cardiovascular disease who do not have hypercholesterolemia"

Russell Ross

2. Aims of the thesis

1. To explore the effect of a regular high-intensity interval training on late luminal loss and the risk of restenosis in patients revascularized by PCI with stent implantation for angina pectoris
2. To evaluate the effect of this training model on aerobic capacity, endothelial function and plasma levels of C-reactive protein and to assess potential correlations between late luminal loss, endothelial function, plasma levels of C-reactive protein and aerobic capacity
3. To assess the effect of a high-intensity interval training program on cardiac autonomic function reflected by time and frequency domain measures of heart rate variability
4. To explore the effect of PCI as a model of mechanical induced plaque rupture on plasma levels of selected inflammatory mediators
5. To evaluate the response of a high-intensity interval training program on levels of markers of inflammation and endothelial cell activation in patients revascularized for coronary artery disease

3. Subjects and Methods

3.1 Study population

Patients referred for planned coronary angiography at the Department of Cardiology, Stavanger University Hospital, were screened for eligibility before angiography.

After successful PCI in one or several native coronary arteries, which was defined as a residual diameter stenosis after stent implantation of less than 20% of the reference diameter, 40 consecutive patients were included. Exclusion criteria included a history of MI or CABG, restenosis after previous PCI, significant valvular heart disease, age over 80 years, inability to give informed consent, inability to participate in regular training due to residency, work situation or comorbidity, any known chronic inflammatory disease other than atherosclerosis, persisting or permanent atrial fibrillation or planned surgery within the next 6 months. Patients were randomized to either a high-intensity interval training program for 6 months or to usual care.

The study was performed according to the Helsinki declaration. The local ethics committee approved the protocol and all patients gave written informed consent.

During the inclusion period 224 patients matched the inclusion criterion “successful PCI” with stenting in a native coronary artery for angina pectoris. Reasons for exclusion were inability to participate in regular training due to residency (to far from the training facility) (n=89), work situation (not able to participate in training sessions in the morning due to job) (n=13) or comorbidity (n=24), history of MI or CABG (n=32), heart failure (n=3), age over 80 years (n=6), malignancy or planned surgery within the next 6 months (n=5), significant valvular heart disease (n=3), known chronic inflammatory disease (n=6) or refusal to participate (n=3).

3.2 Exercise Training

The exercise training program was designed based on recently published data confirming the superior effect of aerobic high-intensity interval training in cooperation with two experienced physical therapists specialised in cardiac rehabilitation (13, 14). The patients exercised in groups of 10, starting 11±4 days after PCI, for one hour three times a week for six months. The training model included 10 minutes of warm-up at 60-70 % of maximal heart rate, followed by four 4-minutes intervals at 80-90 % of maximal heart rate, when patients were riding an ergometric bicycle or were running. Intervals were interrupted by 3 minutes of

active recovery at 60-70 % of maximal heart rate. The interval training period was followed by 5 minutes cool-down, 10 minutes abdominal- and spine-resistance exercises and 5 minutes of stretching and relaxing. Patients were wearing individual pulse watches to monitor the intended target heart rate during training.

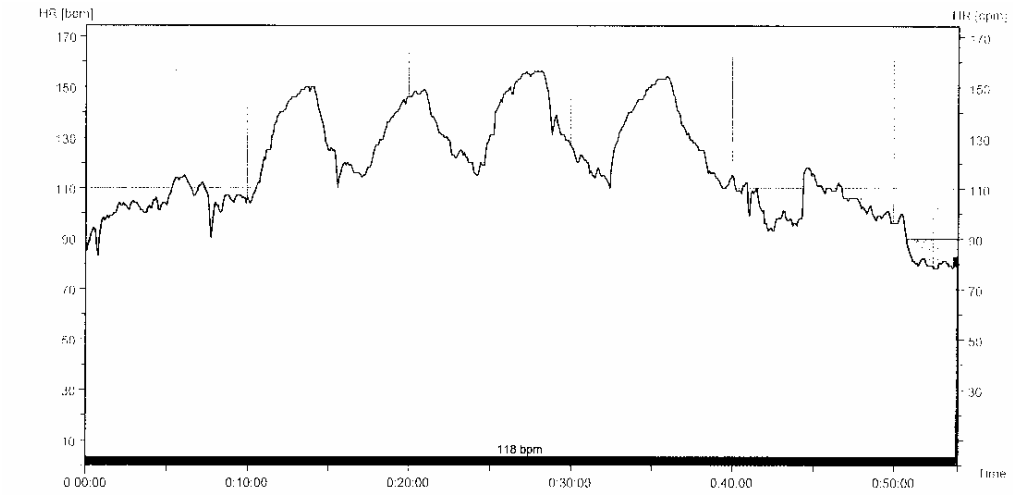

Figure 1. Original pulse registration during interval training showing four pulse peaks.

3.3 Quantitative coronary angiography

Standard image acquisition was performed using 2 or more angiographic projections of the stenosis. Intracoronary nitroglycerin was administered to provide maximum coronary vasodilation. At follow-up, repetition of identical angiographic projections of the lesion were performed. With the contrast-filled injection catheter as the calibration source, quantitative angiographic analysis was performed by use of a validated computer-assisted edge-detection algorithm (General Electric, USA). Lesion length was defined as the axial extent of the lesion that contained a shoulder-to-shoulder lumen reduction by 20%. Selected images for analysis were identified by use of angiographic projections that demonstrated the stenosis in an unforeshortened view, minimized the degree of vessel overlap, and displayed the stenosis in its “sharpest and tightest” view. A 5-mm segment of reference diameter proximal and distal to the stenosis was used to calculate the average reference vessel diameter at baseline, after stent implantation and at follow-up. The target lesion was defined as the stented segment plus a 5

mm segment proximal and distal to the stent. Angiographic follow-up was performed at 6 months after the index procedure unless earlier angiography was indicated due to clinical reasons. Binary angiographic restenosis was defined as the incidence of percent diameter stenosis of ≥50 % at the qualifying angiographic follow-up. Angiographic percent diameter stenosis was defined as (1 - (Minimal lumen diameter (MLD) - reference vessel diameter)) /100. Late luminal loss (LLL) was defined as the MLD immediately after the procedure minus the MLD at 6-month follow-up (figure 3). In patients with multiple stents implanted, a mean LLL per patient was calculated to compare between groups of training and control. Otherwise, the mean LLL per stent was used to compare stent-dependent LLL between groups.

Two cardiologists independently interpreted the images in a blinded manner. Disagreement was resolved by reanalysis and consensus. The intraobserver variability of absolute vessel dimensions for 20 randomly selected measurements was 0.09 mm. The interobserver variability of absolute vessel dimensions was 0.11 mm.

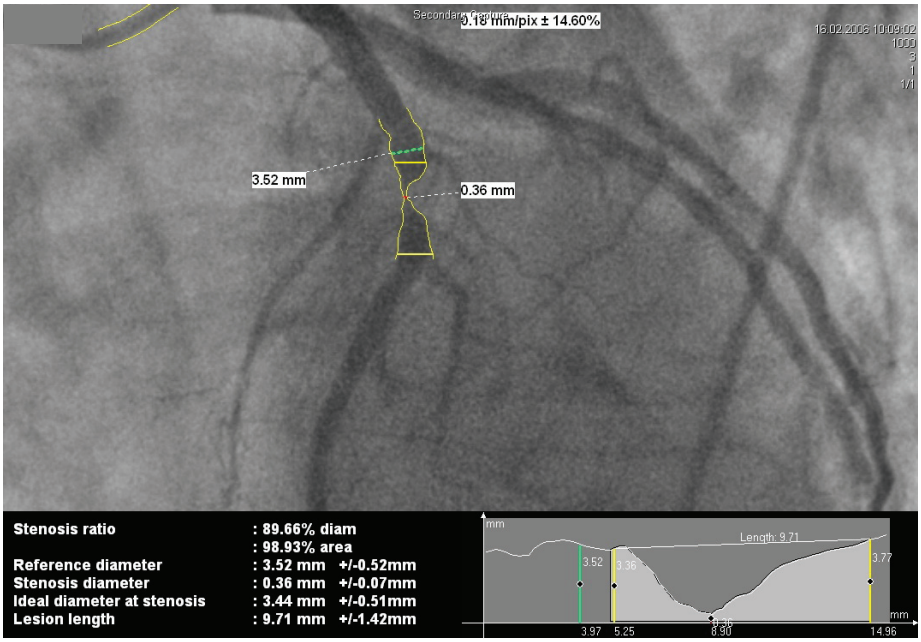

Figure 2. Original registration of a quantitative coronary angiography analysis.

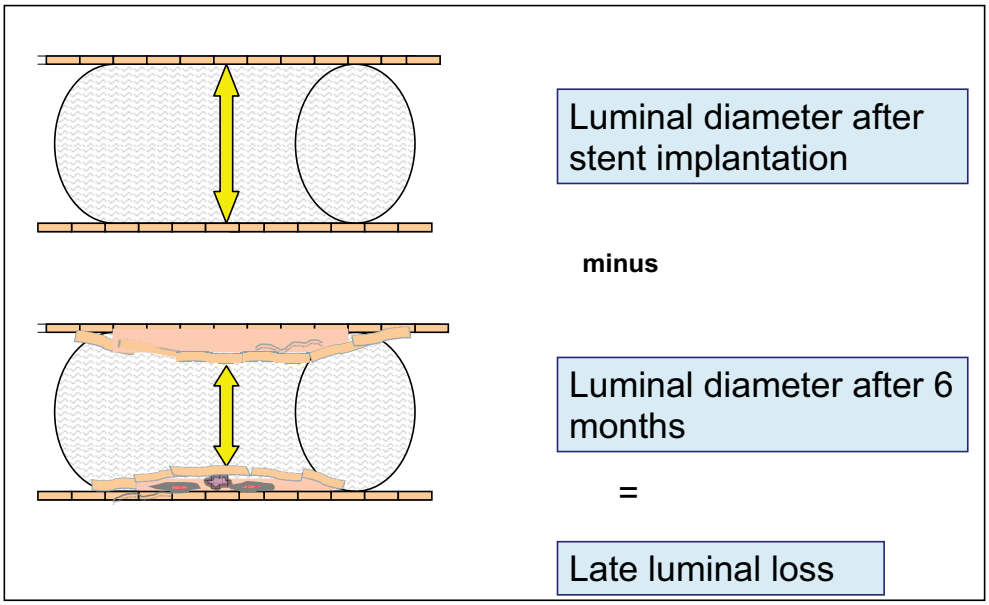

Figure 3. Late luminal loss.

3.4 Aerobic capacity

One week after PCI the subjects performed a symptom-limited ergospirometry to assess safety, to calculate the target heart rate for training and to measure peak oxygen uptake (VO2). Tests were done on an upright, electrically braked cycle ergometer (Model KEM III, Mijnhardt, S.V.Bunnik, The Netherlands) using a 20 Watt/minute ramp protocol. The patients were asked to exercise to exhaustion. Gas exchange data were collected continuously with an automated breath-by-breath system (System 2001, medical Graphics Corporation, St.Paul, Minn., USA).

3.5 Endothelial function

Endothelium-dependent and endothelium-independent dilation of the brachial artery was measured at 7 days and at 6 months after PCI. All examinations were done by the same operator with a 12-MHz ultrasound Doppler probe (Vivid 7 System, GE Vingmed Ultrasound, Horten, Norway), according to the guidelines by Corretti et al. (3). Vasoactive medication was withheld for 48 hours. Imaging of the brachial artery was done above the antecubital fossa in the longitudinal plane. A segment with clear anterior and posterior intimal interfaces between the lumen and the vessel wall was selected for continuous 2D greyscale imaging and

measuring of the diameter before inflation of a pneumatic cuff on the upper arm to 250 mm Hg for 5 minutes. Flow-mediated vasodilation (FMD) was expressed as percentage dilation from baseline diameter to that observed 1 minute after cuff release.

Endothelium-independent dilation was measured 3 minutes after administration of 0.4 mg nitroglycerin sublingually. Five cardiac cycles were analyzed and averaged for each scan. All scans were analyzed twice with EchoPACtm (GE Vingmed Ultrasound) by to blinded investigators.

3.6 Heart Rate Variability

HRV measures were obtained from a 3-channel portable Holter worn for 24 hours as recommended by the task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (32) at baseline (1 week after PCI) and at 6 months. The commercially available MT-200 Holter System from Schiller (Switzerland) with the MT-210 Analysis Software Version 1.0.0 was used to analyse time and frequency domain measures of HRV. In a continuous 24 hours ECG recording, each R wave or QRS complex is detected and labelled as normal sinus (N) or abnormal. The complete 24 hour registrations were carefully edited using visual checks and manual corrections of incorrect designated complexes by an experienced medical technologist kept unaware of study data. Three time periods, 24 hours, daytime (09:00-21:00) and night time (00:00- 06:00) were analyzed. For quality reasons only 5-minute segments of the recording with ≥95% of normal beats were included. Since many time domain measures correlate closely with others, four indices were chosen as recommended by the task force: the standard deviation of all NN intervals in the entire 24 hour registration (SDNN), the triangular interpolation of NN interval histogram (TINN), the standard deviation of the average normal RR intervals for 288 5-minute segments of a 24 hour ECG recording (SDANN) and the root mean square of differences between successive NN intervals (RMSSD).

Additionally, frequency domain analysis was performed using a non-parametric method (fast Fourier transformation). The power spectrum was quantified into various frequency bands as standards (32). The frequency domain indices total-power, ultra low frequency (ULF), very low frequency (VLF), low frequency (LF), high frequency (HF), very high frequency (VHF) and the LV/HF-ratio were reported.

3.7 Markers of inflammation and endothelial cell activation

Blood sampling protocol

Blood samples were collected by standard venipuncture from the left median cubital vein before angiography, immediately after PCI, and 3 hours, 24 hours, 3 days, 7 days, 6 weeks and 6 months after PCI. EDTA (ethylenediaminetetraacetic acid) plasma tubes were immediately stored on ice and separated within 30 minutes by centrifugation at 4º C and 1800*g* for 15 minutes to obtain platelet-poor plasma. Samples were stored at -80º C until analysis and thawed only once.

Biochemical measurements

CRP concentrations were measured by a high-sensitivity particle-enhanced immunoturbidimetric method by the use of Roche Modular P automated clinical chemistry analyzers (Roche Diagnostics, Basel, Switzerland) and reagents of Tina-quant CRP (latex) assay (Roche Diagnostics). Plasma concentrations of IL-6, IL-10, PTX3, TNFα, VCAM-1, E-selectin, P-selectin, MCP-1/CCL2, RANTES/CCL5, CCL19, CCL21, IL-8/CXCL8 and CXCL16 were quantified by enzyme immunoassays (EIAs) obtained from R&D Systems (Minneapolis, MN). Plasma concentrations of vWF were analyzed by EIA using antibodies from DakoCytomation (Glostrup, Denmark) as previously described (33). Plasma concentrations of CD40L were analyzed by EIA provided by Bender MedSystems GmbH (Vienna, Austria). The intra- and inter-assay coefficients of variation were <10% for all assays.

3.8 Statistics

Sample size calculation and randomization

LLL six month after stent implantation is usually between 0 and 0.8 mm (21). In the Exercise Training Intervention After Coronary Angioplasty (ETICA)-trial LLL following PCI was 0.54 mm smaller in the training group compared to the control group (34). Taking into account that 30 to 50% of patients would receive a DES, we chose an estimated difference in mean lumen loss between groups of 0.35 mm. To obtain 80% power for detection of this difference, assuming a standard deviation of 0.4 mm in each group, a sample size of 40 patients was needed.

Block randomization (four blocks of 10 individuals, with equal number of treatments) was used to achieve evenly balanced treatment group numbers at the end of each block. The order of treatments within the block was randomly permuted by a computer-generated sequence.

Statistical analysis

All data were statistically analyzed by SPPS 15.0 (SPSS Inc., Chicago, Illinois). The Kolmogorov-Smirnov test and visual inspection of QQ-plots were used to test for normal distribution of continuous data.

Main outcome data with skewed distribution (late luminal loss, FMD and CRP) are reported as median and interquartile range. Normally distributed data are expressed as mean ± standard deviation (SD) or absolute numbers (n). Skewed data of HRV measures were logarithmically transformed to correct for skewness before statistical analysis. In the analysis of HRV measures a linear regression model was applied to test for treatment effects with adjustment for baseline factors

Comparisons between groups were analyzed by two-sided t-test or Mann-Whitney U test as appropriate. Comparison of categorical variables was generated by the Pearson chi-square test or Fisher`s exact test. Spearman correlation was used to calculate correlation coefficients. Analysis of variance with repeated measures (ANOVA) was used to test for differences of biomarker levels between treatment groups over time. A linear mixed-effect model (R-statistics version 2.9.1.2009) was applied to analyze the changes in plasma levels of inflammatory mediators over time as the dependent variables and fixed effects for stent type, access site and use of glycoprotein IIb/IIIa-inhibitors.

All tests were 2-tailed and a p-value below the 0.05 level was considered significant.

4.Summary of results

4.1 Paper 1

Aim: High-intensity interval training has been shown to be superior to moderate continuous exercise training in improving exercise capacity and endothelial function in patients with coronary artery disease. The objective of this study was to evaluate if this training model could reduce the risk of restenosis measured as late luminal loss following PCI for stable or unstable angina.

Methods and results:

We prospectively randomized 40 patients after successful PCI with implantation of a bare metal stent (n=30) or drug eluting stent (n=32) to a 6 months supervised high-intensity interval exercise training program (n=20) or to a control group (n=20). At six months restenosis, measured as in-segment late luminal loss of the stented coronary area, was smaller in the training group (0.21±0.39 mm) compared to the control group (0.55±0.41 mm, p=0.01). Reduction of late luminal loss in the training group was consistent with both stent types. Peak oxygen uptake increased in the training and control group by 17.6% and 0.5%, respectively ($p<0.01$). Flow-mediated dilation improved 6.5% in the training group and 0.3% in the control group (p=0.01). Levels of high-sensitivity CRP decreased in the training group by 0.8±1.6 mg/l and increased by 0.1±0.9 mg/l in the control group (p=0.03).

Conclusions: Regular high-intensity interval exercise training was associated with a significant reduction in late luminal loss in the stented coronary segment. This effect was associated with increased aerobic capacity, improved endothelium function and attenuated levels of CRP.

4.2 Paper 2

Aim: Low time domain measures of heart rate variability have been shown to predict outcome after myocardial infarction. The predictive value of heart rate variability, when measured in patients with coronary artery disease without myocardial infarction is less clear. Further, little is known about the mechanisms how autonomic imbalance affects outcome.

Methods and results: Forty patients following PCI with stent implantation for angina pectoris were prospectively randomized to a six months supervised high-intensity interval training program (n=20) or to a control group (n=20). All patients underwent 24-hour Holter

monitoring, measurement of peak oxygen uptake and ultrasound assessment of endothelial function at baseline and at six months.

At baseline there were no significant differences between groups. In the training group all time domain indices and the frequency domain indices, total power and ultralow frequency of heart rate variability, increased significantly during the training period. Mean heart rate decreased significantly. In the control group only the root mean square of differences between successive NN intervals (ln RMSSD) increased significantly.

Changes in standard deviations of the average NN intervals (SDANN) and lnRMSSD were significantly correlated to changes in peak oxygen uptake ($R=0.47$ and 0.39; $p<0.01$ and $p=0.03$ respectively). Heart rate variability measures were not significantly correlated to endothelial function.

Conclusions: High-intensity exercise training over 6 months significantly improved heart rate variability measures in patients following PCI with stent implantation. The effect on heart rate variability was correlated to changes in peak oxygen uptake, but not to changes in endothelial function.

4.3 Paper 3

Aim: PCI can be regarded as a model for mechanical induced plaque rupture. The objective of this study was to evaluate the inflammatory response to PCI in stable coronary artery disease by analysing plasma levels of a wide range of inflammatory mediators.

Methods: Consecutively, we included 36 patients with stable angina pectoris after successful revascularization by PCI with implantation of a bare metal stent or drug eluting stent. Patients were followed for 7 days with serial measurements of inflammatory mediators in plasma.

Results: CRP and Pentraxin 3 showed a statistical significant early increase after PCI peaking at 3 days and 3 hours, respectively. Vascular cell adhesion molecule-1 (VCAM-1) increased significantly with a peak at 3 days, while E-selectin showed a statistical significant gradual decrease. Markers of platelet mediated inflammation showed increasing (CD40 ligand) and decreasing (P-selectin) levels after PCI. While monocyte chemoattractant protein, CCL21 and CXCL16 increased rapidly in response to PCI, IL-8, CCL19 and RANTES decreased. Patients with drug eluting stents had significantly lower levels of VCAM-1 and RANTES compared to those with bare metal stents. A femoral access site was associated with higher CRP levels than a radial access site. The use of glycoprotein-IIb/IIIa-inhibitors was associated with significantly higher CD40L and RANTES levels.

Conclusions: Our findings underscore the complex nature of the inflammatory responses during PCI in stable coronary artery disease, and suggest that simultaneous measurements of several markers may be needed to characterize these PCI-related responses. The responses were only in a minor degree influenced by stent type, access site and the use of glycoprotein-IIb/IIIa-inhibitors.

4.4 Paper 4

Aim: Increased plasma levels of inflammatory markers and markers of endothelial cell activation have been associated with increased risk for cardiovascular events. Exercise training may lower the risk for coronary heart disease by attenuating inflammation and improving endothelial function. The objective of this study was to evaluate effects of regular high-intensity exercise training on a wide range of markers of inflammation and endothelial cell activation.

Material and methods: Consecutively, 36 patients were prospectively randomized to a 6 months supervised high-intensity interval training program or to a control group following successful PCI for stable angina pectoris. Blood samples were drawn at baseline (before angiography) and at 6 months.

Results: At 6 months, levels of the inflammatory markers IL-6 and IL-8 were reduced and levels of the anti-inflammatory cytokine IL-10 increased in the training group only. The decrease in IL-6 and CRP levels were significantly correlated with the decrease in luminal loss following PCI. In contrast to these anti-inflammatory effects, training had no effect on markers of platelet-mediated inflammation, and the effect of training on markers on endothelial cell activation were rather complex showing attenuating (von Willebrand factor) and enhancing (E-selectin and vascular cell adhesion molecule 1) effects.

Conclusions: Regular exercise training in stable angina patients following PCI may attenuate some, but not all, inflammatory pathways, potentially contributing to the beneficial effects of exercise training on restenosis.

5. General discussion

In paper 1 we showed that a high-intensity aerobic interval training model following PCI with stent implantation for angina pectoris was associated with significant reduced LLL suggesting that this training program is associated with a lower risk of restenosis. Reduced LLL in the training group was seen in patients with both BMS and DES. This effect was accompanied by significant improvements in aerobic capacity with higher peak oxygen uptake and a higher anaerobic threshold in the training group. Further, endothelial function measured as brachial artery reactivity improved and levels of CRP decreased significantly in the training group only.

In paper 2 we assessed the effect of this training model on cardiac autonomic function reflected by both time domain and frequency domain measures of heart rate variability. We found that all time domain indices and the frequency domain indices, total power and ultralow frequency of heart rate variability, increased significantly in the training group during the training period. At the same time mean heart rate decreased significantly. In the control group only the root mean square of differences between successive NN intervals (ln RMSSD) increased significantly.
The increase in SDANN and lnRMSSD were significantly positively correlated to improvements in peak oxygen uptake.

In paper 3 we characterized the inflammatory response to PCI with stent implantation in thirty-six patients with stable angina, who were followed for 7 days with serial measurements of inflammatory mediators in plasma. We observed a complex reaction of markers of inflammation that underscore the complex nature of the inflammatory responses during PCI. The responses were only in a minor degree influenced by stent type, access site and the use of glycoprotein-IIb/IIIa-inhibitors.

In paper 4 we further elucidated the effect of training on inflammation by expanding the analysis to a wide range of inflammatory markers and makers of endothelial cell activation. The results indicate that regular exercise training in CAD patients following PCI was associated with an anti-inflammatory effect reflected by significant reductions of plasma levels of IL-6, IL8 and an increase of IL-10.

The changes of plasma levels of CRP and IL-6 were significantly correlated to LLL at 6 months, suggesting that this anti-inflammatory effect contributes to the reduction of LLL. Training had no effect on markers of platelet-mediated inflammation, and the effect of training on markers on endothelial cell activation were rather complex showing attenuating (von Willebrand factor) and enhancing (E-selectin and vascular cell adhesion molecule 1) effects.

5.1 Restenosis

The only previous trial, who has explored the effect of training on restenosis is the ETICA trial by Bellardinelli et al (34). The results of the current study are consistent with the results of the ETICA trial, which found lower residual diameter stenosis and fewer cardiac events in the training group. The rapid progression of the treatment of CAD in the past years have set limitations to the ETICA trial as it was conducted before the statin era and only approximately two thirds of the patients received a stent. Both stent implantation and statin treatment have been shown to reduce the restenosis rate (35, 36). Most patients in the current study have been on treatment with statins for several weeks prior to PCI. Our results indicate that a high-intensity exercise training program for 6 months after PCI with stent implantation might reduce the risk of in-stent restenosis in a well-treated population with angina pectoris. The mechanisms of restenosis are linked to endothelial dysfunction and inflammation. The high-intensity interval training program induced reduction of late luminal loss may be associated with improvements in these processes

5.2 Endothelial function

There was a significant training-induced improvement in endothelial dependent brachial artery reactivity in the current study. The relationship between endothelial dysfunction in the brachial artery as assessed by ultrasound imaging and endothelial dysfunction noted on coronary angiography after acetylcholine injection is well documented (37).

Low endothelial shear stress modulates endothelial gene expression through complex mechanoreception and mechanotransduction processes, inducing an atherogenic endothelial phenotype (38). In arterial regions with disturbed laminar flow low endothelial shear stress attenuates nitric oxide (NO)-dependent atheroprotection (39), promotes low-density lipoprotein cholesterol uptake by endothelial cells (40), promotes oxidative stress (39) and inflammation (41), promotes vascular smooth muscle cell migration, differentiation and

proliferation (42) and degradation of extracellular matrix in vascular wall and plaque fibrous cap (43).

Regular exercise training increases endothelial shear stress by increasing the spatial blood gradient of blood velocity at the vessel wall and has the potential to reverse the pro-atherogenic effects associated with low shear forces.

Hambrecht et al. have shown that endurance training improves endothelium-dependent vasodilatation in coronary arteries by activating endothelial NO-synthase, resulting in increased levels of NO in coronary endothelial cells (44). Localized delivered NO may also be an inhibitor of neointima formation as shown in an animal carotid balloon injury model (45), suggesting a potential mechanistic effect of training on LLL. Further, endothelial dysfunction has been used as a surrogate of atherosclerotic disease activity (46) and is an independent predictor of cardiovascular events and prognosis (47). In addition, impaired FMD early after PCI has been shown to predict the occurrence of in-stent restenosis in a prospective study (48), indicating a link between endothelial dysfunction and neointimal growth.

5.3 Inflammatory markers and markers of endothelial cell activation

Large epidemiological studies have shown an inverse relationship between physical activity and inflammation (49-51) suggesting that attenuated systemic inflammation may be one of the mechanisms through which physical activity leads to a reduction in cardiovascular risk.

Current American guidelines have implied CRP as a risk marker and conclude that it is reasonable to measure hs-CRP as an adjunct to the major risk factors to further assess absolute risk for coronary disease primary prevention (52). These recommendations are based on CRPs ability to independently predict the risk of cardiovascular events (53). Further, serum-levels of CRP before PCI (54) and the response of CRP to PCI have been shown to be associated with restenosis (19). The reduction of hs-CRP levels and IL-6 in the training group reported in the first and fourth paper indicates an anti-inflammatory effect of regular training. This modulation of the inflammatory profile may be operative for the training induced reduction of LLL as reductions of plasma levels of both CRP and IL-6 were associated with less LLL at 6 months.

The attenuation of inflammation in the training group is in accordance with previous studies showing a training induced reduction in the inflammatory response both in patients with CAD and heart failure (55, 56). Previous studies have also documented that CRP decreases NO production and increases endothelin-1 production by endothelial cells leading to endothelial dysfunction (57).

Experimental studies using transgenic mouse models indicate a cause response relationship between CRP and atherosclerotic disease (58). Recently, Xing et al. demonstrated that neointima formation after vascular injury is exaggerated in human CRP transgenic mice (59), implying an potential role for CRP in the development of restenosis.

In paper 4 we elucidate the effect of long-term training on inflammation and expanded the analysis to selected markers of inflammation and markers of endothelial cell activation. We found that the training group, but not the control group, showed a significant decrease in plasma levels of the inflammatory mediators IL-6, IL-8 and CCL21 as well as an increase in plasma levels of the anti-inflammatory cytokine IL-10, and as for IL-6 and IL-8, the difference in changes between the two study groups reached statistical significance.

The changes in IL-6 and CRP levels were significantly correlated with LLL following PCI, suggesting a possible link between the effect of aerobic interval training on restenosis following PCI and the anti-inflammatory net effects of such physical activity.

Previous studies examining the effect of exercise training on markers of inflammation in different populations with cardiovascular risk factors have been diverging, and several of these studies were not randomized (60-69). In a study comparing PCI with exercise training, regular physical exercise training was associated with a significant reduction of both inflammatory markers and ischemic events (70). In addition, Peschel et al. found that high-frequency and duration exercise training reduced the expression of adhesion molecules on monocytes in stable CAD patients, while a less intensive home-based program did not (71), indicating that the intensity of the training program matters with regard to the anti-inflammatory effect. However, although training and PCI have been compared, no previous randomized studies have prospectively examined the effect of exercise training on inflammation in CAD patients following PCI. Here, we show a significant difference in changes in plasma levels of the prototypical inflammatory cytokines IL-6 and IL-8 between the training and the control group, with a significant decrease in these cytokines during exercise training, accompanied by an increase in the anti-inflammatory cytokine IL-10. Our findings further support an anti-inflammatory potential of exercise training in CAD patients, including the follow-up period after PCI.

The decrease in IL-8 levels, being a pro-atherogenic chemokine, may be of interest, and notably, the regulation of IL-8 seems to be closely related to oxidative stress and hypoxia, that could have been attenuated by training. The decrease of both IL-6 and CRP levels supports that aerobic interval training attenuates the inflammatory IL-6-CRP pathway. Plasma levels of IL-6 are strongly associated with future cardiac events and mortality in patients with

stable CAD (72), and peri-procedural plasma CRP and IL-6-levels have been associated with the risk of restenosis (19, 73). IL-6 has been shown to promote vascular smooth muscle cell proliferation, and our findings in the present study, showing a strong correlation between the decrease in plasma levels of IL-6 and the reduction of late luminal loss in the training group following PCI, may further relate IL-6 to the restenosis process, a process that seems to be attenuated by exercise training.

While most studies on the effects of exercise training on inflammation in CAD patients have been focused on a few central mediators, we in the present randomized trial examined a wide range of inflammatory mediators. Thus, while training had attenuating effects on some central inflammatory markers (i.e., IL-6 and IL-8), aerobic interval training had no effect on markers of platelet-mediated inflammation. Moreover, whereas training decreased IL-8 and CCL21 levels, plasma concentration of most of the chemokines that were examined (i.e., RANTES, MCP-1, CXCL16, CCL19) did not change during the training period. Finally, whereas exercise training has been shown to improve endothelial function in CAD patients, our findings in the current study may suggest a more complex effect of training on endothelial cells, inducing both attenuating (i.e. decreased vWF levels) and enhancing (i.e., increased VCAM-1 and E-selectin levels) effects on markers of endothelial cell activation.

5.4 Aerobic capacity

Exercise capacity measured as VO_2 consumption is the strongest independent predictor of all-cause and cardiovascular mortality compared with other established risk factors in patients with cardiovascular disease (74). The substantial increase both in peak VO_2 (17.6 %), anaerobic threshold (23.3 %) and maximal work load (17.2%) in the training group is comparable with the results from previous studies applying a high-intensity interval training program (12). Exercise training has therefore a potential beneficial prognostic effect in addition to the reduction in in-stent restenosis rate in this patient population.

5.5 Cardiac autonomic function

Coronary artery disease is associated with cardiac autonomic imbalance reflected by low heart rate variability (75). Low HRV has been shown to predict mortality after myocardial infarction (76) and is a risk marker for cardiac events in the general population (77). The predictive value of HRV for myocardial infarction or cardiovascular mortality, when measured in patients with angina pectoris without previous myocardial infarction is not clear.

Myocardial infarction and heart failure lead to activation of the sympathetic and the renin-angiotensine-aldosterone system, which both depress HRV (78, 79). Previous MI and congestive heart failure were thus exclusion criteria for this study.

The indices SDNN and TINN are estimates of overall HRV. SDANN reflects long-term components of HRV mediated by both sympathetic and parasympathetic influences, while RMSSD reflects short-term components of HRV mediated by parasympathetic respiratory variations.

After 6 months of HIIT HRV was significantly improved reflected by an increase in all time domain indices and several frequency domain measures. The increase in time domain measures was consistent at day-and night time. The observed increase in SDNN of 27 ms or 22% was much larger than the average increase in SDNN of 9 ms or 16% seen in a current published meta-analysis (31) and might be the direct effect of this efficient training program over a period of six months.

The improvement in the time domain indices SDANN and rMSSD were significantly correlated to an increase in physical fitness, but not to improvements in endothelial function. The significant reduction in mean heart rate and increase in rMSSD in the training group indicates a shift towards increased parasympathetic and decreased sympathetic nervous activity.

The evaluation of the effect of training on HRV in randomized controlled trials is limited by the heterogeneity of patient populations included and methods used to measure HRV (31). The present study differs from these trials in that patients after MI and congestive heart failure had been excluded and a modern high-intensity interval training program was applied, that seem to be more effective compared to a traditionally moderate continuously intensity program in improving aerobic exercise capacity and endothelial function in different patient populations (12-14).

However, this potential beneficial effect is limited by the fact that there is no sufficient evidence to determine whether changes in HRV indices translates into clinical benefit nor what can be regarded a clinically significant improvement in HRV.

5.6 Effects of training

Regular high-intensity training has multifactorial effects in patients with CAD including improved risk factor profile. Exercise training as part of a lifestyle-intervention has been shown to attenuate the progression of coronary stenosis (80-82). However, the regression of coronary plaques was comparatively small and makes it difficult to explain the substantial

increase of myocardial perfusion seen on scintigraphy (83) and the risk reduction in cardiac events in these trials (81). Improvement of myocardial perfusion may be achieved independently from regression of stenotic lesions mediated by improved endothelial function. By increasing local endothelial shear stress regular exercise training has the potential to shift endothelial function and structure to an anti-atherosclerotic phenotype attenuating formation and progression of atherosclerotic plaques and vascular remodelling.

5.7 Limitations

Although this randomized, controlled evaluation of the effect of high-intensity interval training demonstrated favourable angiographic and clinical outcomes in the training arm, one should be careful when interpreting the results due to the small sample size and rather large standard deviations. A further limitation is the heterogeneity of stents implanted. There are differences between different types of BMS or DES with regard to the degree of LLL that might have influenced the results. However, the training effect was consistent in both the BMS and the DES patients. Moreover, the present study was performed in patients with angina pectoris following PCI, and this situation may not necessarily be extrapolated to the general CAD population. The actual patients had moderate CAD with a modest inflammatory load. The inflammatory response to the PCI with stent implantation and the subsequent reparative process may overwhelm the inflammatory response of the underlying atherosclerotic process which in turn may explain the modest findings on inflammatory markers. In addition the patients were all on statins which are known to have potent anti-inflammatory properties.

6. Conclusions and implications

1. Our results imply that a high-intensity interval training program may reduce in-stent restenosis following PCI with stenting. Further, training resulted in a marked increase in aerobic capacity, work load and improved endothelial function, which are known to be associated with a more favourable prognosis.

2. A six months high-intensity interval training program results in favourable effects on cardiac autonomic function. However, if these effects translate into clinical benefits is currently not known. A large prospective study evaluating HRV with regard to intervention is needed to develop a robust prediction model that can be used in clinical practice.

3. PCI and stenting can be regarded as a model for mechanical induced plaque rupture. The trauma to the vessel wall leads to a complex response of circulating plasma levels of markers of inflammation and endothelial cell activation. Simultaneous measurements of several markers may be needed to characterize these PCI-related responses. Forthcoming studies should investigate if the inclusion of several of these markers will give more prognostic information than the measurement of standard inflammatory markers such as CRP.

4. In clinical stable patients revascularized with PCI and on optimal medical treatment a 6 months high-intensity interval training program attenuates some, but not all, inflammatory pathways, potentially contributing to the beneficial effects of exercise training on restenosis.

The findings are hypothesis generating for larger randomised trials in this population. If the results on restenosis can be confirmed in larger studies, high-intensity interval training programs should become a natural part of the treatment program for these patients.

7. References

1. Suaya JA, Shepard DS, Normand SL, Ades PA, Prottas J, Stason WB. Use of cardiac rehabilitation by Medicare beneficiaries after myocardial infarction or coronary bypass surgery. *Circulation* 2007; 116(15):1653-1662.
2. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005; 352(16):1685-1695.
3. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002; 39(2):257-265.
4. Murray CJ, Lopez AD. Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. *Lancet* 1997; 349(9063):1436-1442.
5. Ulset E, Undheim R, Malterud K. [Has the obesity epidemic reached Norway?]. *Tidsskr Nor Laegeforen* 2007; 127(1):34-37.
6. Boule NG, Haddad E, Kenny GP, Wells GA, Sigal RJ. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *Jama* 2001; 286(10):1218-1227.
7. Halbert JA, Silagy CA, Finucane P, Withers RT, Hamdorf PA, Andrews GR. The effectiveness of exercise training in lowering blood pressure: a meta-analysis of randomised controlled trials of 4 weeks or longer. *J Hum Hypertens* 1997; 11(10):641-649.
8. Heldal M, Sire S. Effects of intensive exercise training on lipid levels in high risk post-MI patients. *Eur Heart J* 1994; 15(10):1362-1367.
9. Balady GJ, Williams MA, Ades PA, Bittner V, Comoss P, Foody JM, Franklin B, Sanderson B, Southard D. Core components of cardiac rehabilitation/secondary prevention programs: 2007 update: a scientific statement from the American Heart Association Exercise, Cardiac Rehabilitation, and Prevention Committee, the Council on Clinical Cardiology; the Councils on Cardiovascular Nursing, Epidemiology and Prevention, and Nutrition, Physical Activity, and Metabolism; and the American Association of Cardiovascular and Pulmonary Rehabilitation. *Circulation* 2007; 115(20):2675-2682.
10. Taylor RS, Brown A, Ebrahim S, Jolliffe J, Noorani H, Rees K, Skidmore B, Stone JA, Thompson DR, Oldridge N. Exercise-based rehabilitation for patients with coronary heart disease: systematic review and meta-analysis of randomized controlled trials. *Am J Med* 2004; 116(10):682-692.
11. Piepoli MF, Davos C, Francis DP, Coats AJ. Exercise training meta-analysis of trials in patients with chronic heart failure (ExTraMATCH). *Bmj* 2004; 328(7433):189.
12. Rognmo O, Hetland E, Helgerud J, Hoff J, Slordahl SA. High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease. *Eur J Cardiovasc Prev Rehabil* 2004; 11(3):216-222.
13. Tjonna AE, Lee SJ, Rognmo O, Stolen TO, Bye A, Haram PM, Loennechen JP, Al-Share QY, Skogvoll E, Slordahl SA, Kemi OJ, Najjar SM, Wisloff U. Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. *Circulation* 2008; 118(4):346-354.
14. Wisloff U, Stoylen A, Loennechen JP, Bruvold M, Rognmo O, Haram PM, Tjonna AE, Helgerud J, Slordahl SA, Lee SJ, Videm V, Bye A, Smith GL, Najjar SM, Ellingsen O, Skjaerpe T. Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation* 2007; 115(24):3086-3094.

15. Mauri L, Orav EJ, Kuntz RE. Late loss in lumen diameter and binary restenosis for drug-eluting stent comparison. *Circulation* 2005; 111(25):3435-3442.
16. Post MJ, de Smet BJ, van der Helm Y, Borst C, Kuntz RE. Arterial remodeling after balloon angioplasty or stenting in an atherosclerotic experimental model. *Circulation* 1997; 96(3):996-1003.
17. Lowe HC, Oesterle SN, Khachigian LM. Coronary in-stent restenosis: current status and future strategies. *J Am Coll Cardiol* 2002; 39(2):183-193.
18. Wentzel JJ, Krams R, Schuurbiers JC, Oomen JA, Kloet J, van Der Giessen WJ, Serruys PW, Slager CJ. Relationship between neointimal thickness and shear stress after Wallstent implantation in human coronary arteries. *Circulation* 2001; 103(13):1740-1745.
19. Saleh N, Tornvall P. Serum C-reactive protein response to percutaneous coronary intervention in patients with unstable or stable angina pectoris is associated with the risk of clinical restenosis. *Atherosclerosis* 2007; 195(2):374-378.
20. van Tiel CM, Bonta PI, Rittersma SZ, Beijk MA, Bradley EJ, Klous AM, Koch KT, Baas F, Jukema JW, Pons D, Sampietro ML, Pannekoek H, de Winter RJ, de Vries CJ. p27kip1-838C>A single nucleotide polymorphism is associated with restenosis risk after coronary stenting and modulates p27kip1 promoter activity. *Circulation* 2009; 120(8):669-676.
21. Morice MC, Serruys PW, Sousa JE, Fajadet J, Ban Hayashi E, Perin M, Colombo A, Schuler G, Barragan P, Guagliumi G, Molnar F, Falotico R. A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. *N Engl J Med* 2002; 346(23):1773-1780.
22. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W, Jr., Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation* 1995; 92(5):1355-1374.
23. Jonasson L, Holm J, Skalli O, Bondjers G, Hansson GK. Regional accumulations of T cells, macrophages, and smooth muscle cells in the human atherosclerotic plaque. *Arteriosclerosis* 1986; 6(2):131-138.
24. Kovanen PT, Kaartinen M, Paavonen T. Infiltrates of activated mast cells at the site of coronary atheromatous erosion or rupture in myocardial infarction. *Circulation* 1995; 92(5):1084-1088.
25. Saleh N, Svane B, Hansson LO, Jensen J, Nilsson T, Danielsson O, Tornvall P. Response of serum C-reactive protein to percutaneous coronary intervention has prognostic value. *Clin Chem* 2005; 51(11):2124-2130.
26. Almagor M, Keren A, Banai S. Increased C-reactive protein level after coronary stent implantation in patients with stable coronary artery disease. *Am Heart J* 2003; 145(2):248-253.
27. Bonz AW, Lengenfelder B, Jacobs M, Strotmann J, Held S, Ertl G, Voelker W. Cytokine response after percutaneous coronary intervention in stable angina: effect of selective glycoprotein IIb/IIIa receptor antagonism. *Am Heart J* 2003; 145(4):693-699.
28. Taschenatlas der Physiologie. Stefan Silbernagl, Agamemnon Despopoulos. Thieme 1983, ISBN 3135677028.
29. Tsuji H, Larson MG, Venditti FJ, Jr., Manders ES, Evans JC, Feldman CL, Levy D. Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation* 1996; 94(11):2850-2855.
30. Levy WC, Cerqueira MD, Harp GD, Johannessen KA, Abrass IB, Schwartz RS, Stratton JR. Effect of endurance exercise training on heart rate variability at rest in healthy young and older men. *Am J Cardiol* 1998; 82(10):1236-1241.

31. Nolan RP, Jong P, Barry-Bianchi SM, Tanaka TH, Floras JS. Effects of drug, biobehavioral and exercise therapies on heart rate variability in coronary artery disease: a systematic review. *Eur J Cardiovasc Prev Rehabil* 2008; 15(4):386-396.
32. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Eur Heart J* 1996; 17(3):354-381.
33. Bollerslev J, Ueland T, Jorgensen AP, Fougner KJ, Wergeland R, Schreiner T, Burman P. Positive effects of a physiological dose of GH on markers of atherogenesis: a placebo-controlled study in patients with adult-onset GH deficiency. *Eur J Endocrinol* 2006; 154(4):537-543.
34. Belardinelli R, Paolini I, Cianci G, Piva R, Georgiou D, Purcaro A. Exercise training intervention after coronary angioplasty: the ETICA trial. *J Am Coll Cardiol* 2001; 37(7):1891-1900.
35. Serruys PW, Kutryk MJ, Ong AT. Coronary-artery stents. *N Engl J Med* 2006; 354(5):483-495.
36. Walter DH, Schachinger V, Elsner M, Mach S, Auch-Schwelk W, Zeiher AM. Effect of statin therapy on restenosis after coronary stent implantation. *Am J Cardiol* 2000; 85(8):962-968.
37. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC, et al. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol* 1995; 26(5):1235-1241.
38. Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL, Stone PH. Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. *J Am Coll Cardiol* 2007; 49(25):2379-2393.
39. Harrison DG, Widder J, Grumbach I, Chen W, Weber M, Searles C. Endothelial mechanotransduction, nitric oxide and vascular inflammation. *J Intern Med* 2006; 259(4):351-363.
40. Gimbrone MA, Jr., Topper JN, Nagel T, Anderson KR, Garcia-Cardena G. Endothelial dysfunction, hemodynamic forces, and atherogenesis. *Ann N Y Acad Sci* 2000; 902:230-239; discussion 239-240.
41. Nagel T, Resnick N, Dewey CF, Jr., Gimbrone MA, Jr. Vascular endothelial cells respond to spatial gradients in fluid shear stress by enhanced activation of transcription factors. *Arterioscler Thromb Vasc Biol* 1999; 19(8):1825-1834.
42. Malek AM, Alper SL, Izumo S. Hemodynamic shear stress and its role in atherosclerosis. *Jama* 1999; 282(21):2035-2042.
43. Cheng C, Tempel D, van Haperen R, van der Baan A, Grosveld F, Daemen MJ, Krams R, de Crom R. Atherosclerotic lesion size and vulnerability are determined by patterns of fluid shear stress. *Circulation* 2006; 113(23):2744-2753.
44. Hambrecht R, Adams V, Erbs S, Linke A, Krankel N, Shu Y, Baither Y, Gielen S, Thiele H, Gummert JF, Mohr FW, Schuler G. Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. *Circulation* 2003; 107(25):3152-3158.
45. Lipke EA, West JL. Localized delivery of nitric oxide from hydrogels inhibits neointima formation in a rat carotid balloon injury model. *Acta Biomater* 2005; 1(6):597-606.
46. Willerson JT, Kereiakes DJ. Endothelial dysfunction. *Circulation* 2003; 108(17):2060-2061.
47. Halcox JP, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, Nour KR, Quyyumi AA. Prognostic value of coronary vascular endothelial dysfunction. *Circulation* 2002; 106(6):653-658.

48. Patti G, Pasceri V, Melfi R, Goffredo C, Chello M, D'Ambrosio A, Montesanti R, Di Sciascio G. Impaired flow-mediated dilation and risk of restenosis in patients undergoing coronary stent implantation. *Circulation* 2005; 111(1):70-75.
49. Mora S, Cook N, Buring JE, Ridker PM, Lee IM. Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms. *Circulation* 2007; 116(19):2110-2118.
50. Ford ES. Does exercise reduce inflammation? Physical activity and C-reactive protein among U.S. adults. *Epidemiology* 2002; 13(5):561-568.
51. Boekholdt SM, Sandhu MS, Day NE, Luben R, Bingham SA, Peters RJ, Wareham NJ, Khaw KT. Physical activity, C-reactive protein levels and the risk of future coronary artery disease in apparently healthy men and women: the EPIC-Norfolk prospective population study. *Eur J Cardiovasc Prev Rehabil* 2006; 13(6):970-976.
52. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, 3rd, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC, Jr., Taubert K, Tracy RP, Vinicor F. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003; 107(3):499-511.
53. Ridker PM. C-reactive protein and the prediction of cardiovascular events among those at intermediate risk: moving an inflammatory hypothesis toward consensus. *J Am Coll Cardiol* 2007; 49(21):2129-2138.
54. Ferrante G, Niccoli G, Biasucci LM, Liuzzo G, Burzotta F, Galiuto L, Trani C, Rebuzzi AG, Crea F. Association between C-reactive protein and angiographic restenosis after bare metal stents: an updated and comprehensive meta-analysis of 2747 patients. *Cardiovasc Revasc Med* 2008; 9(3):156-165.
55. Goldhammer E, Tanchilevitch A, Maor I, Beniamini Y, Rosenschein U, Sagiv M. Exercise training modulates cytokines activity in coronary heart disease patients. *Int J Cardiol* 2005; 100(1):93-99.
56. Adamopoulos S, Parissis J, Kroupis C, Georgiadis M, Karatzas D, Karavolias G, Koniavitou K, Coats AJ, Kremastinos DT. Physical training reduces peripheral markers of inflammation in patients with chronic heart failure. *Eur Heart J* 2001; 22(9):791-797.
57. Venugopal SK, Devaraj S, Yuhanna I, Shaul P, Jialal I. Demonstration that C-reactive protein decreases eNOS expression and bioactivity in human aortic endothelial cells. *Circulation* 2002; 106(12):1439-1441.
58. Danenberg HD, Szalai AJ, Swaminathan RV, Peng L, Chen Z, Seifert P, Fay WP, Simon DI, Edelman ER. Increased thrombosis after arterial injury in human C-reactive protein-transgenic mice. *Circulation* 2003; 108(5):512-515.
59. Xing D, Hage FG, Chen YF, McCrory MA, Feng W, Skibinski GA, Majid-Hassan E, Oparil S, Szalai AJ. Exaggerated neointima formation in human C-reactive protein transgenic mice is IgG Fc receptor type I (Fc gamma RI)-dependent. *Am J Pathol* 2008; 172(1):22-30.
60. Nicklas BJ, Hsu FC, Brinkley TJ, Church T, Goodpaster BH, Kritchevsky SB, Pahor M. Exercise training and plasma C-reactive protein and interleukin-6 in elderly people. *J Am Geriatr Soc* 2008; 56(11):2045-2052.
61. Timmerman KL, Flynn MG, Coen PM, Markofski MM, Pence BD. Exercise training-induced lowering of inflammatory (CD14+CD16+) monocytes: a role in the anti-inflammatory influence of exercise? *J Leukoc Biol* 2008; 84(5):1271-1278.
62. Stewart LK, Flynn MG, Campbell WW, Craig BA, Robinson JP, Timmerman KL, McFarlin BK, Coen PM, Talbert E. The influence of exercise training on inflammatory cytokines and C-reactive protein. *Med Sci Sports Exerc* 2007; 39(10):1714-1719.

63. Zoppini G, Targher G, Zamboni C, Venturi C, Cacciatori V, Moghetti P, Muggeo M. Effects of moderate-intensity exercise training on plasma biomarkers of inflammation and endothelial dysfunction in older patients with type 2 diabetes. *Nutr Metab Cardiovasc Dis* 2006; 16(8):543-549.
64. Oberbach A, Tonjes A, Kloting N, Fasshauer M, Kratzsch J, Busse MW, Paschke R, Stumvoll M, Bluher M. Effect of a 4 week physical training program on plasma concentrations of inflammatory markers in patients with abnormal glucose tolerance. *Eur J Endocrinol* 2006; 154(4):577-585.
65. Niessner A, Richter B, Penka M, Steiner S, Strasser B, Ziegler S, Heeb-Elze E, Zorn G, Leitner-Heinschink A, Niessner C, Wojta J, Huber K. Endurance training reduces circulating inflammatory markers in persons at risk of coronary events: impact on plaque stabilization? *Atherosclerosis* 2006; 186(1):160-165.
66. Marcell TJ, McAuley KA, Traustadottir T, Reaven PD. Exercise training is not associated with improved levels of C-reactive protein or adiponectin. *Metabolism* 2005; 54(4):533-541.
67. Okita K, Nishijima H, Murakami T, Nagai T, Morita N, Yonezawa K, Iizuka K, Kawaguchi H, Kitabatake A. Can exercise training with weight loss lower serum C-reactive protein levels? *Arterioscler Thromb Vasc Biol* 2004; 24(10):1868-1873.
68. Mattusch F, Dufaux B, Heine O, Mertens I, Rost R. Reduction of the plasma concentration of C-reactive protein following nine months of endurance training. *Int J Sports Med* 2000; 21(1):21-24.
69. Hammett CJ, Prapavessis H, Baldi JC, Varo N, Schoenbeck U, Ameratunga R, French JK, White HD, Stewart RA. Effects of exercise training on 5 inflammatory markers associated with cardiovascular risk. *Am Heart J* 2006; 151(2):367 e367-367 e316.
70. Walther C, Mobius-Winkler S, Linke A, Bruegel M, Thiery J, Schuler G, Halbrecht R. Regular exercise training compared with percutaneous intervention leads to a reduction of inflammatory markers and cardiovascular events in patients with coronary artery disease. *Eur J Cardiovasc Prev Rehabil* 2008; 15(1):107-112.
71. Peschel T, Sixt S, Beitz F, Sonnabend M, Muth G, Thiele H, Tarnok A, Schuler G, Niebauer J. High, but not moderate frequency and duration of exercise training induces downregulation of the expression of inflammatory and atherogenic adhesion molecules. *Eur J Cardiovasc Prev Rehabil* 2007; 14(3):476-482.
72. Fisman EZ, Benderly M, Esper RJ, Behar S, Boyko V, Adler Y, Tanne D, Matas Z, Tenenbaum A. Interleukin-6 and the risk of future cardiovascular events in patients with angina pectoris and/or healed myocardial infarction. *Am J Cardiol* 2006; 98(1):14-18.
73. Ezhov MV, Sumarokov AB, Raimbekova IR, Masenko VP, Naumov VG. Interleukin 6 but not interleukin 10 is associated with restenosis after coronary stenting. *Atherosclerosis* 2003; 169(1):193-194.
74. Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood JE. Exercise capacity and mortality among men referred for exercise testing. *N Engl J Med* 2002; 346(11):793-801.
75. Airaksinen KE, Ikaheimo MJ, Linnaluoto MK, Niemela M, Takkunen JT. Impaired vagal heart rate control in coronary artery disease. *Br Heart J* 1987; 58(6):592-597.
76. La Rovere MT, Bigger JT, Jr., Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. *Lancet* 1998; 351(9101):478-484.
77. Iellamo F, Legramante JM, Massaro M, Raimondi G, Galante A. Effects of a residential exercise training on baroreflex sensitivity and heart rate variability in patients with coronary artery disease: A randomized, controlled study. *Circulation* 2000; 102(21):2588-2592.

78. Bigger JT, Jr., Fleiss JL, Steinman RC, Rolnitzky LM, Kleiger RE, Rottman JN. Correlations among time and frequency domain measures of heart period variability two weeks after acute myocardial infarction. *Am J Cardiol* 1992; 69(9):891-898.
79. Larsen AI, Gjesdal K, Hall C, Aukrust P, Aarsland T, Dickstein K. Effect of exercise training in patients with heart failure: a pilot study on autonomic balance assessed by heart rate variability. *Eur J Cardiovasc Prev Rehabil* 2004; 11(2):162-167.
80. Ornish D, Scherwitz LW, Billings JH, Brown SE, Gould KL, Merritt TA, Sparler S, Armstrong WT, Ports TA, Kirkeeide RL, Hogeboom C, Brand RJ. Intensive lifestyle changes for reversal of coronary heart disease. *Jama* 1998; 280(23):2001-2007.
81. Haskell WL, Alderman EL, Fair JM, Maron DJ, Mackey SF, Superko HR, Williams PT, Johnstone IM, Champagne MA, Krauss RM, et al. Effects of intensive multiple risk factor reduction on coronary atherosclerosis and clinical cardiac events in men and women with coronary artery disease. The Stanford Coronary Risk Intervention Project (SCRIP). *Circulation* 1994; 89(3):975-990.
82. Schuler G, Hambrecht R, Schlierf G, Niebauer J, Hauer K, Neumann J, Hoberg E, Drinkmann A, Bacher F, Grunze M, et al. Regular physical exercise and low-fat diet. Effects on progression of coronary artery disease. *Circulation* 1992; 86(1):1-11.
83. Schuler G, Hambrecht R, Schlierf G, Grunze M, Methfessel S, Hauer K, Kubler W. Myocardial perfusion and regression of coronary artery disease in patients on a regimen of intensive physical exercise and low fat diet. *J Am Coll Cardiol* 1992; 19(1):34-42.

8. Errata

Paper 2:

Table 1, correct number of patients with unstable angina in training group is 3(15).

Paper 2:

Discussion, "... to be associated with an activation of the sympathetic and the renin-angiotensin~~e~~-aldosterone system, which depress~~es~~ HRV [7,8].

Paper 4:

Results, plasma levels of chemokines, last sentence

"…and MCP-1 did not change significantly during follow-up in neither group~~s~~ (Table 2)."