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Exercise-induced effects on coronary atherosclerosis assessed by grayscale and radiofrequency intravascular ultrasound

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Doctoral Thesis

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Thesis for the degree of Philosophiae Doctor

Trondheim, November 2015

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"With respect to the treatment of this complaint, I have little or nothing to advance (...)

I knew one who set himself a task of sawing wood for half an hour every day, and was nearly cured".

William Heberden. Some account of a disorder of the breast.
Medical Transactions 2, 59-67 (1772) London: Royal College of Physicians.

Effektar av uthaldstrening på koronar aterosklerose vurdert med gråskala og radiofrekvens intravaskulær ultralyd

Aterosklerose i koronararteriene er ei leiande årsak til sjukdom og død. Fysisk inaktivitet er ein uavhengig risikofaktor for koronar hjertesjukdom, og det er vist at pasientar som er fysisk aktive har bedre prognose enn inaktive pasientar. Sjølv om det er ein klar klinisk samanheng mellom fysisk aktivitet og hjertehelse, er mange patofysiologiske effektar av fysisk trening i hjertet og kransarteriene ukjende. Dette arbeidet består av tre delstudiar som baserer seg på ein randomisert, kontrollert studie av 36 pasientar og ein tverrsnittstudie av 15 pasientar. Alle pasientane hadde etablert koronarsjukdom og vart behandla med implantasjon av stent.

Målet for studie 1 var å undersøke om uthaldstrening kunne føre til fordelaktige endringar i koronare plakk, og om det var skilnad mellom intervalltrening med høg intensitet og uthaldstrening med moderat intensitet. Gråskala og radiofrekvens intravaskulær ultralyd vart brukt til å vurdere studiens endepunkt. Vi fann ein moderat reduksjon av nekrotisk plakkjerne og total plakkmengde ved oppfølging, på høvesvis om lag 3 og 10 prosent. Det var ingen skilnad mellom dei to treningsgruppene. Desse resultata støttar hypotesen om at uthaldstrening påverkar koronar aterosklerose på ein gunstig måte.

I studie 2 undersøkte vi om kliniske variablar ved studieinklusjon kunne assosierast til reduksjon av nekrotisk plakkjerne eller total plakkmengde ved oppfølging. Vi fann ein sterk og signifikant assosiasjon mellom den kliniske presentasjonen av koronarsjukdommen og nekrotisk plakkjerne volum ved oppfølging. Assosiasjonen var til fordel for pasientar med stabil koronarsjukdom samanlikna med ustabilt koronarsyndrom. Resultata frå denne studien gir grunnlag for ein hypotese om at dei positive effektane av uthaldstrening på koronare plakk kan vere ulik mellom pasientar med stabil koronarsjukdom og ustabilt koronarsyndrom.

I studie 3 undersøkte vi kor godt samsvar det var mellom to ulike undersøkingar med intravaskulær ultralyd i samme pasient på same tidspunkt (reproduserbarheit). Vi fann at det var svært godt samsvar mellom undersøkingane i dei delane av arterien som ikkje var stenta (relativ skilnad mellom to undersøkingar under 5 prosent), medan det var noko dårlegare samsvar mellom undersøkingane i den delen av arterien som var stenta (relativ skilnad mellom 5 og 10 prosent). Desse resultata kan får betyding for framtidige studier som brukar denne metoden.

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Contents

Papers

Acknowledgements

This thesis is based on studies conducted between 2010-2013 at the Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, and the Department of Cardiology, St.Olavs University Hospital.

I received a PhD grant from the Liaison Committee for Central Norway Regional Health Authority and the Norwegian University of Science and Technology in 2009, and have been a resident at the Department of Cardiology, the Department of Medicine and Department of Pulmonary Medicine, St.Olavs University Hospital since 2011. The studies received funding by the Research Fund at St.Olavs University Hospital, the Norwegian Council on Cardiovascular Disease, and the Norwegian Council for Public Health.

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Erik Madssen

Trondheim, April 24th 2015

Errata

Paper I

Text on page 1508 (just below Figure 5)

"3 patients in the MCT group (patient numbers 10, 24, and 37, Figure 4)" should be replaced with

"2 patients in MCT groups (patient numbers 10 and 37, Figure 4)"

List of papers included in the thesis

Paper I

Madssen E, Moholdt T, Videm V, Wisløff U, Hegbom K, Wiseth R. Coronary Atheroma Regression and Plaque Characteristics Assessed by Grayscale and Radiofrequency Intravascular Ultrasound After Aerobic Exercise. *Am J Cardiol* 2014;114:1504-11.

Paper II

Madssen E, Videm V, Moholdt T, Wisløff U, Hegbom K, Wiseth R. Predictors of beneficial coronary plaque changes following aerobic exercise. *Med Sci Sports Exerc.* 2015; Mar 31, epub ahead of print, DOI: 10.1249/MSS.0000000000000672

Paper III

Madssen E, Jakala J, Proniewska K, Kulaga T, Hegbom K, Wiseth R. Reproducibility of grayscale and radiogfrequency IVUS data acquisition in stented coronary arteries. *Scand Cardiovasc J* 2014;48:284-90.

Abbreviations

ACS	Acute coronary syndrome
AIT	Aerobic interval training
CAD	Coronary artery disease
CVD	Cardiovascular disease
GS-IVUS	Grayscale intravascular ultrasound
IVUS	Intravascular ultrasound
MCT	Moderate continuous training
NSTE-ACS	Non-ST elevation acute coronary syndrome
NSTEMI	Non-ST elevation myocardial infarction
PCI	Percutaneous coronary intervention
RF-IVUS	Radiofrequency intravascular ultrasound
SCAD	Stable coronary artery disease
VO_{2max}	Maximal oxygen uptake
VO_{2peak}	Peak oxygen uptake

Summary of thesis

Coronary atherosclerosis is a leading cause of morbidity and mortality worldwide. Physical inactivity is an independent risk factor for coronary artery disease, and physically active patients have an improved prognosis compared to sedate patients. Although the clinical relationship between exercise and cardiovascular health is convincing, the pathophysiological mechanisms responsible for improved outcomes in this large patient group are largely unknown.

In a randomized controlled trial, we assessed exercise-induced effects on coronary atherosclerosis in 36 patients with established coronary artery disease undergoing stent implantation and optimal medical therapy. Patients exercised for 12 weeks, either following an aerobic interval training protocol, or performing moderate continuous exercise. Coronary atherosclerosis was assessed by grayscale and radiofrequency intravascular ultrasound, quantifying both plaque geometry and tissue characteristics. Our main findings were that with both exercise protocols there was a significant reduction in necrotic core (≈ 3 %) and a strong trend towards a reduction of plaque burden (≈ 10 %) in non-stented coronary segments and in separate atheroma lesions. There were no differences between exercise groups.

In a post-hoc analysis of data from the randomized controlled trial, we assessed clinical factors at baseline that potentially were associated with a reduction in necrotic core and plaque burden at follow-up. We found a strong association between the clinical presentation of disease and necrotic core volume reduction after aerobic exercise (p=0.011). The association was in favor of patients with stable coronary artery disease, and necrotic core volume reduction was much more frequent in these patients (17/18) than in patients with unstable coronary artery disease (8/18). There were no significant associations between any clinical baseline explanatory variables and plaque burden reduction at follow-up.

In a third study conducted within a subgroup of patients included in the randomized controlled trial, we assessed the reproducibility of intravascular ultrasound data acquisition in stented coronary arteries. This was performed by repeating the intravascular ultrasound pullback twice at the same time-point, thus simulating data collection in a serial imaging study. Our main finding was that the inter-pullback reproducibility of geometrical data was very good for non-stented segments with relative differences between pullbacks < 5 %. For stented segments reproducibility was poorer, though acceptable, and < 10 %.

Taken together, the data presented in this thesis strengthens the scientific evidence for beneficial exercise-induced effects on coronary atherosclerosis, not only with respect to atherosclerotic burden, but also with respect to plaque vulnerability. Furthermore, exercise-induced effects on coronary atherosclerosis may be more beneficial in patients with stable coronary artery disease compared to patients in the early phase after an acute coronary syndrome. Finally, serial intravascular ultrasound imaging in stented coronary arteries seems to be associated with a variability of 5–10 % attributed to the acquisition of images itself, which may have implications for the design of future serial stent studies.

1 Introduction

1.1 Coronary artery disease

Coronary artery disease (CAD) is the most common cause of heart disease and the single most prevalent cause of death in the developed world [1]. Mortality rates from CAD and cardiovascular disease (CVD) in general are declining in affluent countries [2], but continues to rise in in low- and middle-income countries [3-5]. CAD is projected to remain the leading cause of death worldwide for the indefinite future [6].

In 2012, CVD accounted for approximately 13 000 deaths in Norway [7], and CAD was the single most important cause within this group (Figure 1). In 2013, a total of 13 043 myocardial infarctions in 12 336 patients were recorded in the Norwegian Myocardial Infarction Registry [8]. Of these, 28 % were classified as ST-elevation myocardial infarctions and 70 % were classified as non-ST-elevation myocardial infarctions (NSTEMI). The 30-day mortality in the entire population was approximately 10 %.
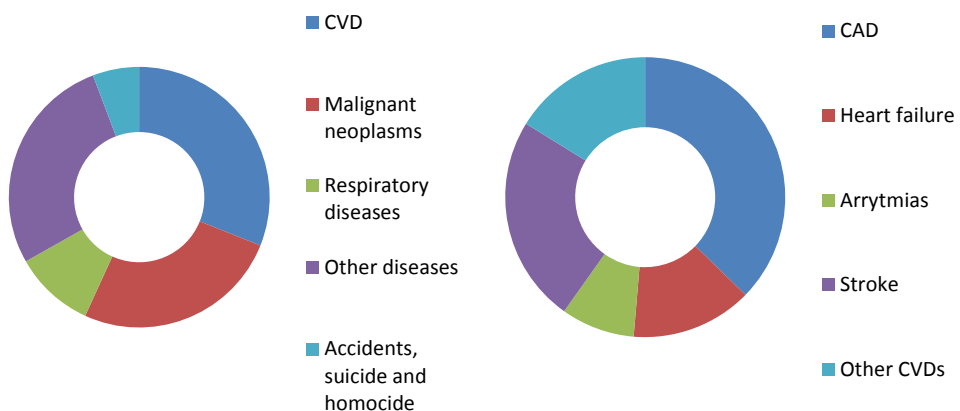
Figure 1. Causes of deaths in Norway 2012 (left), and etiology of deaths from CVD in Norway 2012 (right). Pie charts based on data from Statistics Norway [7].

Non-modifiable risk factors for CAD include a genetic predisposition, age, and male gender. CAD is also strongly associated with life-style factors, such as obesity, hypertension, hyperglycemia, hypercholesterolemia, tobacco use, unhealthy diet, and physical inactivity[9-15]. Many of these modifiable risk factors are closely associated through joint pathophysiological mechanisms that include low-grade chronic inflammation (see 1.1.1), endothelial dysfunction, and insulin resistance.

Population-based strategies aiming to reduce the CVD risk burden have been successful in many countries with respect to tobacco use. However, recent studies show that the prevalence of obesity and physical inactivity is high [16,17], and increasing [18]. Thus, new strategies in the prevention of CAD and CVDs need to target the fact that populations are gaining weight and become less physically active.

1.1.1 Pathophysiology of atherosclerosis

The vast majority of coronary artery disease is caused by coronary atherosclerosis. Atherosclerosis originates mainly at sites of bifurcation or branching points in the arterial system, which are areas where endothelial shear stress is low or oscillating [19], and therefore favours transport of blood components across the vessel wall. Atherosclerosis can be defined as a chronic inflammatory condition initiated in the endothelium in response to injury, and maintained through the interactions between modified lipoproteins, particularly low-density lipoprotein cholesterol, T-lymphocytes, monocyte-derived macrophages, and the normal constituents of the arterial wall [20]. Atherosclerosis develops from the normal intima, to atheroma precursors, and finally to advanced atherosclerotic lesions, depending on the presence of cardiovascular risk factors and chronic inflammation.

Endothelial dysfunction is thought to represent the initial stage of atherosclerosis [21]. The normal endothelium maintains vasodilatory properties of arteries, regulating vascular

homeostasis, and inhibiting inflammation, cell adhesion and intravascular coagulation [22]. When exposed to disturbances in endothelial shear stress (see 1.3.2), biomechanical forces from hypertension and the presence of other cardiovascular risk factors, the endothelium loses its normal properties. Endothelial actions shift towards vasoconstriction and dysregulation of vascular homeostasis, a phenomenon named endothelial dysfunction[23].

An increased expression of cell adhesion molecules and inflammatory mediators [24] on the endothelial surface occurs in the dysfunctional endothelium, allowing different leukocytes to enter the arterial wall [25]. In the intima, monocytes differentiate into macrophages [26], and a subendothelial retention of lipids [27], particularly low-density lipoproteins, initiates chronic inflammation [28]. Low-density lipoproteins are subjected to oxidation and enzymatic modification, giving rise to oxidized low-density lipoproteins, which promotes atherosclerosis (Figure 2)[29].
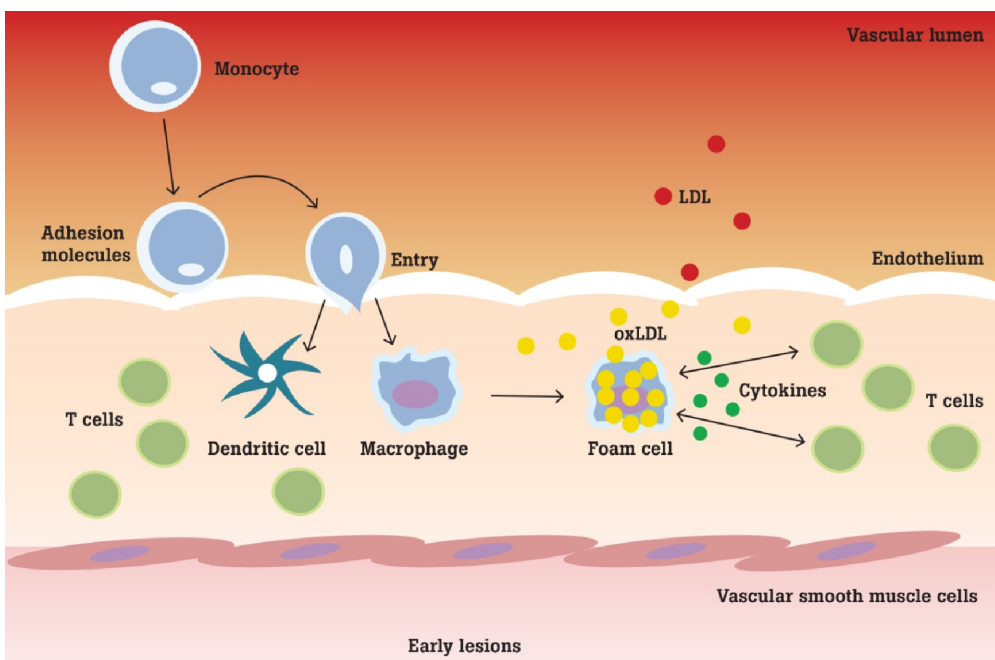
Figure 2. Vascular inflammation and development of early atheromas. See text for details. LDL; low-density lipoprotein, oxLDL; oxidized low-density lipoprotein. Figure designed by Chris Eliassen on request.

Macrophages engulf lipids from the subendothelial space and take the appearance of foamy structures, called foam cells. These cells release growth factors and cytokines which stimulate migration and proliferation of smooth muscle cells and myofibroblasts, and formation of a fibrous cap covering the atheroma follows. Further progression of atherosclerosis is characterized by increased lipid retention and inflammation. This development is driven by interactions between different subsets of activated macrophages and T-lymphocytes [26,30]. The M1 macrophage, which inhibits tissue proliferation and causes tissue damage[31], has recently been identified as important with respect to the development of unstable plaques [32].

1.1.2 Coronary atheromas

The main components of atherosclerotic plaques are connective tissue (extracellular matrix, collagen, proteoglycans and elastic fibers), cholesterol and phospholipids, immune cells, smooth muscle cells and thrombotic material [27]. Different variations of these components give rise to a variety of heterogeneous lesions with different clinical significance, which are summarized in Table 1 [33-35].

There are three basic mechanisms that give rise to coronary thrombosis [32], which is the cause of rapid coronary occlusion giving rise to acute coronary syndromes (ACS). Plaque rupture of a vulnerable coronary plaque is the most common mechanism and is present in approximately 70 % of cases. Plaque erosion [27], due to loss or dysfunction of the lumen endothelial cells, is the principal mechanism in 25-30 % of cases, while calcified nodule is a rare cause of coronary thrombosis related to disruptive nodular calcifications protruding into the coronary lumen.

The dynamic balance between the synthesis of collagen and degradation in the cap of the plaque determines the plaque's fragility, and thereby the risk of rupture or erosion (plaque

vulnerability) [36]. Generally, type I, II and III lesions (Table 1) are asymptomatic, while type IV lesions are stable plaques, which may clinically present as angina pectoris when the coronary stenosis reaches a certain level. Type V lesions, and particularly when a thin and inflamed cap is present (thin-capped fibroatheroma), are considered unstable plaques.

Lesion type	Characteristics	Note
Type I	Increased numbers of macrophages, filled with lipid droplets, appear as foam cells.	Represent the very initial changes. These lesions are also found in children.
Type II	Layers of foam cells within layers of smooth muscle cells.	"Fatty streak", the first macroscopically visible lesion.
Type III	Pools of extracellular lipids.	"Pathological intimal thickening" and represent transition to atheromas.
Type IV	Lipid core of extracellular cholesterol and phospholipids intermixed with normal intima.	Atheromas
Type V	Va: Lipid core covered by an acquired fibrous cap. Vb: Increased extent of calcification within the lesion. Vc: Increased extent of fibrous tissue within the lesion.	Va are fibroathermoas or thin capped fibroatheromas. Vb lesions are calcific lesions. Vc lesions are fibrous lesions.
Type VI	Acute complicated type IV and V lesions with rupture or erosion.	Leading to different clinical entities depending on the nature of the thrombosis.

Table 1. Different morphological lesions types of coronary plaques with characteristics.

1.1.3 Diagnosis and treatment of coronary artery disease

Atherosclerosis is usually a silent disease for decades, until an acute event or a gradual obstruction of the artery lumen causes clinical symptoms. The clinical presentations are heterogeneous, and include silent ischemia (no symptoms), stable angina pectoris (no symptoms at rest), unstable angina pectoris (symptoms at rest or in light activity, but no

pathological concentrations of troponin in blood), myocardial infarction (with or without changes in the electrogradiogram, but always with troponin concentrations above the cut-off level for normal values), or sudden death.

ACS is a common designation for unstable angina pectoris and myocardial infarction combined. The biomarker troponin and the electrocardiogram are the most important diagnostic tools in the initial assessment of patients with suspected ACS, and determinative for further treatment (Figure 3). Patients included in this thesis were diagnosed with stable coronary artery disease (SCAD, equivalent to angina pectoris) and non-ST-elevation ACS (NSTE-ACS, including unstable angina pectoris and NSTEMI). These conditions are therefore further discussed below.
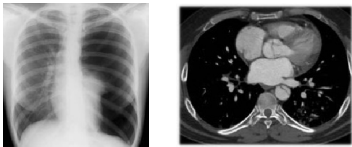
Differential diagnostics in suspected acute coronary syndrome	
Rule out: • Pulmonary embolus • Aortic dissection • Pneumothorax	
ECG with ST-elevation or LBBB	**ST-elevation acute myocardial infarction (STEMI)**
ECG without ST-elevation or LBBB • Troponin rise and fall • Troponin normal	 **Non-ST-elevation acute myocardial infarction (NSTEMI)** **Unstable angina pectoris (or other cause)**

Figure 3. Differential diagnostics in suspected ACS. LBBB; left bundle branch block. Modified from ESC Guidelines [37].

SCAD is caused by a gradual and progressive narrowing of one or several coronary arteries. In situations where there is an increased demand of blood to the myocardium (increased work load), the arterial narrowing causes an imbalance between the need and supply of oxygenated blood. Thus, the most typical feature of SCAD is that patients are symptomatic during activity and symptom-free at rest when the need for oxygenated blood is relatively low. SCAD is diagnosed on the basis of symptoms (most often chest pain, functional dyspnea, fatigue) and findings in non-invasive tests (Table 2) and/or coronary angiography (section 1.2.1).

Test	Diagnosis of coronary artery disease	
	Sensitivity (%)	Specificity (%)
Exercise electrocardiogram	45-50	85-90
Exercise stress echocardiography	80-85	80-88
Exercise stress single photon emission computed tomography	73-92	63-87
Dobutamin stress echocardiography	79-83	92-95
Dobutamin stress magnetic resonance imaging	79-88	81-91
Vasodilator stress echocardiography	72-79	92-95
Vasodilator stress single photon emission computed tomography	90-91	75-84
Vasodilator stress magnetic resonance imaging	67-94	61-85
Coronary computed tomography angiography	95-99	64-83
Vasodilator stress positron emission tomography	81-97	74-91

Table 2. Characteristics of tests used to diagnose coronary artery disease. Modified from ESC Guidelines [38].

All patients with SCAD should have an anti-platelet agent and be considered for anti-ischemic therapy, such as beta-blockers and vasodilatory drugs. Treatment of co-existing risk factors for CAD, such as hypercholesterolemia and hypertension, is also mandatory. The main indication for coronary revascularization is symptoms despite optimal medical therapy [39]. The choice between percutaneous coronary intervention (PCI) and surgical revascularization depends on the extent of CAD (assessed by coronary angiography) and co-morbidity.

NSTE-ACS is a medical emergency and all patients should be hospitalized. Pathological findings in the electrocardiogram may be present or not. Since platelet activation and aggregation play a pivotal role in arterial thrombosis formation, all patients should receive a double anti-platelet regimen at admission, which usually includes acetylsalicylic acid and an inhibitor of adenosinephosphate-mediated platelet aggregation. Anticoagulants are also used to inhibit thrombin generation. Anti-ischemic treatment such as betablockers and/or nitroglycerin is given when indicated, and early treatment with statins is recommended. Guidelines recommend evaluation with coronary angiography and, if indicated, coronary revascularization within 72 hours after the onset of symptoms in most patients [37].

1.2 Invasive imaging of coronary artery disease

There has been a tremendous development in imaging modalities for CAD over the past decades, making it feasible to assess coronary atherosclerosis in vivo on a detailed level that previously only was available post mortem. These improvements have been utilized by clinicians to provide better patient care, and have also given new insight into coronary biology.

1.2.1 Coronary angiography

The angiogram is considered the gold standard in the diagnostics of CAD [38,40], and is used to determine whether a patient has significant disease (i.e. ≈ > 50 % diameter reduction of the artery lumen) and the patient's suitability for percutaneous or surgical revascularization. The procedure is done in local anesthesia with the patient awake. A catheter is introduced into the radial or femoral artery, and X-ray images are taken of both coronary arteries from different

angles to visualize all segments as a planar silhouette of the lumen. A contrast medium is injected intracoronary for each view that is taken. The most common complication associated with angiography is local bleeding, which most often is treated with compression and bed rest. Contrast nephropathy is less common after the introduction of new and less toxic contrast agents. Major cardiovascular events and fatal outcomes are rare [41].

Measurement of fractional flow reserve (FFR) is performed during coronary angiography, and is considered the gold standard for invasive assessment of ischemia [40]. FFR of coronary arteries is defined as the maximum coronary artery flow in the presence of a stenosis divided by the theoretical normal maximum flow of the same artery. Measurement of FFR is based on pressure recordings distal and proximal to the stenosis during maximal vasodilation with adenosine. FFR < 0.75-0.80 indicates a hemodynamic significant coronary stenosis. FFR measurement is used in situations where the indication for treatment based on the angiogram itself is not conclusive [42].

1.2.2 Intravascular ultrasound

Coronary angiography has some major limitations, the most important being underestimation of true disease severity due to diffuse coronary atherosclerosis or coronary remodeling [43]. Intravascular ultrasound (IVUS) overcomes some of these limitations, as it visualizes the full thickness of the vessel wall in cross-sectional images. The IVUS equipment consists of a catheter with an ultrasound transducer and a console, and the method is always used in association with coronary angiography.

The reconstructed image is formed by the amplitude of the reflected ultrasound waves from tissue components in the artery wall, which are converted to electrical signals and sent to an external processing system for reconstruction (Figure 4) [44]. The brightest echo is given from the intima bordering the lumen, and from the border between the external media and

external elastic membrane boarding the adventitia. A motorized pullback with constant

pullback speed is used, and therefore, distances between the cross-sectional images are known

and one may calculate distances, areas and volumes of vessels, stents and plaques. Thus,

IVUS enables an assessment of both the extent and severity of coronary atherosclerosis.

IVUS is limited with respect to different plaque components due to its low spatial and

axial resolution [45]. Characterization of plaques by IVUS is therefore based on visual

appearance alone, and is displayed in black and white, so-called gray scale, GS-IVUS. Thus,

differentiating between for instance a thrombus and a lipid-rich plaque may be difficult due to

the fact that the echoes reflected from these tissue components are similar. Another limitation

of IVUS is the tendency of the catheter to stick or stall during pullbacks due to friction

between the catheter and the vessel. In these situations the reflected image may contain false

dimensions. This issue is further discussed in section 1.2.4 and in Paper 3.
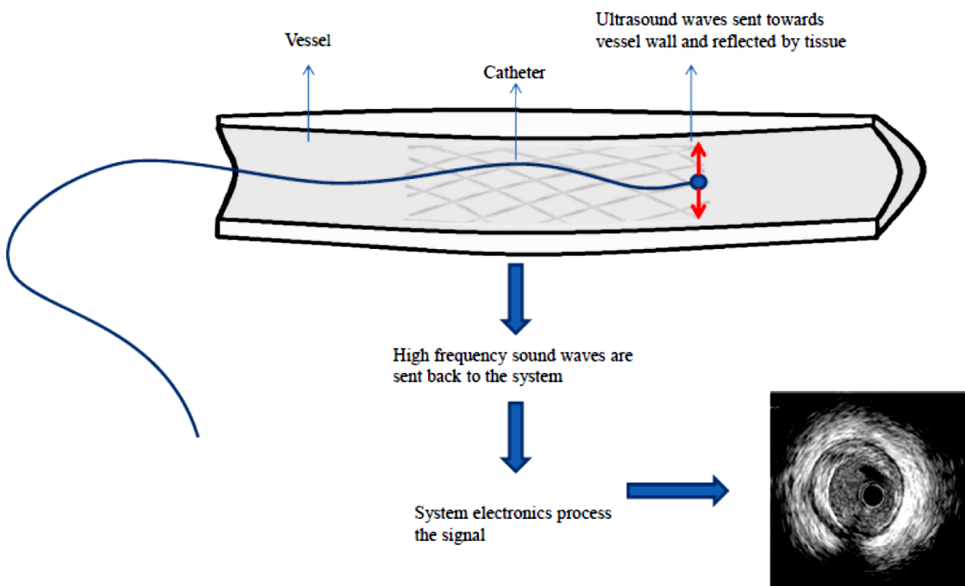
Figure 4. Schematic overview of acquisition of intravascular ultrasound data.

For clinical purposes, IVUS is recommended in selected patients to optimize stent implantation, to assess severity and to optimize treatment of unprotected left main lesions, and to assess mechanisms of stent failure [40]. Relative contraindications for IVUS are small arteries (diameter < 1.5 mm), severe vessel tortuosity, and old venous grafts with general atherosclerosis [46].

1.2.3 Radiofrequency IVUS-based plaque classification

Several post-processing methods have been developed to further characterize tissue and atherosclerotic lesions. Radiofrequency analysis (RF-IVUS) or spectral analysis of intravascular ultrasound data, also called intravascular ultrasound virtual histology (Figure 5), uses the underlying frequency of the reflected ultrasound waves to analyze tissue components [47,48]. RF-IVUS has been correlated with vascular tissue determined from histology with high accuracy [49,50], and characterizes four different vascular tissue types based on the spectral signature of the reflected sound waves; fibrous tissue, fibro-fatty tissue, dense calcium and necrotic core (Figure 5).
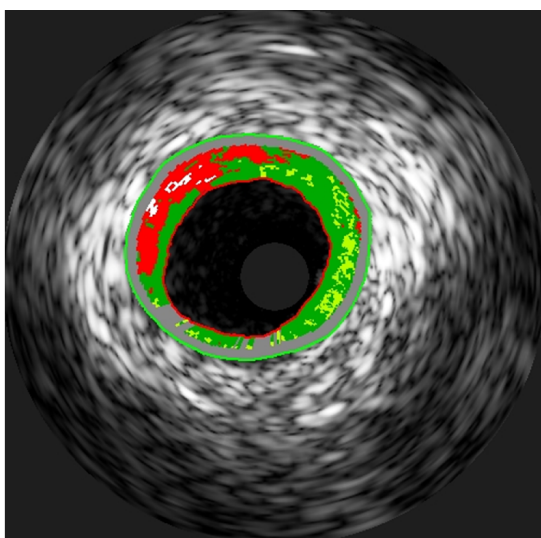
Figure 5. Assessment of a plaque in the left descending coronary artery using RF-IVUS. Different color-codes are used to identify different tissue types. Green = fibrous tissue, green – yellowish = fibro-fatty tissue, white = dense calcium, and red = necrotic core.

Based on pathological data, six different lesion types based on RF-IVUS criteria have been proposed (Table 3), with an additional hypothesis of evolutionary order and degree of vulnerability. This order suggests that the fibrocalcific plaque is the most advanced, and the virtual histology thin cap fibroatheroma is the most vulnerable plaque [51].

Lesion type	Description
Intimal medial thickening	<600 μm of intima thickness
Pathological intimal thickening	≥600 μm thickness for >20 % of the circumference with fibro-fatty tissue > 15 %, and no confluent necrotic core or dense calcium
Fibrotic plaque	Dominant fibro-fatty tissue and no confluent necrotic core or dense calcium
Fibrocalcific plaque	>10 % confluent dense calcium with no confluent necrotic core
Fibroatheroma	>10 % confluent necrotic core on three consecutive frames
Virtual histology thin cap fibroatheroma	>10 % confluent necrotic core on three consecutive frames and arc of necrotic core in contact with the lumen for 36 degrees along lumen circumference

Table 3. Classification of RF-IVUS lesions types, with proposed increased vulnerability from top to bottom in the table, adapted from Garcia-Garcia et al [51]. FT; fibrous tissue, FF; fibro-fatty tissue, NC; necrotic core, and DC; dence calcium.

The Providing Regional Observations to Study Predictors of Events in the Coronary Tree (PROSPECT) investigation followed 697 patients with ACS with a median follow-up of 3.4 years [52]. All patients included in the study underwent GS- and RF-IVUS of all three epicardial vessels and major cardiovascular events during follow-up were adjudicated to either originally treated lesions (culprit) or untreated lesions. The main finding of this study was that acute events during follow up were equally attributable to recurrence of disease at culprit lesions and to non-culprit lesions. Furthermore, non-culprit lesions that were responsible for events were frequently angiographically mild, and characterized as thin-capped fibroatheromas or with a large plaque burden [52]. These findings are important as they

tell us that not only plaque geometry or plaque size, but also plaque composition, is pivotal in plaque risk assessment [53]. Whether coronary plaques being defined as vulnerable by RF-IVUS or other imaging modalities (see 1.2.5) should be invasively treated is the subject of ongoing trials.

1.2.4 Reproducibility of IVUS data

As in any methodology, the reproducibility of both image acquisition and data analysis are pivotal issues when working with IVUS. The issue of reproducibility may be particularly important with respect to IVUS, as changes in plaque characteristics are generally small in serial clinical trials.

Several studies have documented that the analysis of IVUS measurements is highly reproducible, both in non-stented [54] and stented coronary segments [55-57]. The reproducibility of IVUS data acquisition is also found acceptable in non-stented segments [58,59]. However, there is a paucity of data on accuracy between repeated pullbacks from intervened vessels. Implanted stents may represent a particular problem due to a higher tendency of the IVUS catheter to stick or stall during pullbacks through these segments. Variability in data acquisition from stented coronary segments may therefore represent a source of error that could have implications for the design of serial stent studies.

1.2.5 Other imaging modalities

The most established non-invasive imaging modality for CAD is coronary computed tomography angiography. The method visualizes coronary calcification, lumen narrowing and to some degree plaque composition. It is limited by false positive results in the presence of

severe calcification or motion artefacts [60], and is recommended as an alternative to an invasive strategy in the diagnosis of SCAD in patients with low to moderate risk [38].

Optical coherence tomography (OCT) is an invasive method that uses near-infrared light to produce cross-sectional images of coronary arteries. The method has a very high resolution and may therefore be used to study various morphological features of CAD [61], including struts of implanted stents. It is limited by its weak ability to detect vulnerable plaques. OCT is currently being recommended in the assessment of stent failure and to optimize stent implantation [40].

Near-infrared-spectroscopy (NIRS) is a novel method for invasively detection of lipid content in plaques [62] and is currently only being recommended for use in research. Images are displayed as a chemogram, which shows the scanned artery segment as a map, and the probability of lipid is displayed in a color code from red (low probability) to yellow (high probability).

1.3 Physical activity in coronary artery disease

The association between physical activity and reduced risk of CAD has been known for many decades [63], and confirmed in several studies, also in a Norwegian population [64]. Physical inactivity is identified as an independent risk factor for CVD [65] and estimations propose that one fifth of CVDs are related to by physical inactivity [66]. Inactivity is also closely related to the development of obesity, diabetes mellitus and hypertension, which all represent independent cardiovascular risk factors. It is therefore not surprising that physical exercise is associated with reduced morbidity and mortality in subjects with established CVDs [67-69]. In fact, a recent meta-analysis showed no detectable differences between exercise and drug intervention in the secondary prophylaxis of CAD [70], emphasizing the importance of "prescribing" physical activity as treatment equally to cardiovascular drugs in this patient

group. The mechanisms responsible for the favorable effects of physical activity in CAD

patients are not completely understood, and may include both modification of CAD risk

factors [71] and a direct effect on coronary atherosclerosis.

1.3.1 Aerobic fitness and exercise intensity

Aerobic fitness or exercise capacity is defined by the maximal oxygen uptake (VO_{2max}),

which is the highest uptake of oxygen during strenuous exercise using large muscle groups.

VO_{2max} is defined as the product of cardiac output and arteriovenous oxygen difference

(Fick's equation):

$$\boxed{VO_{2max} = (\text{Heart rate x stroke volume}) \times (\text{Arterial}O_2 \text{. Venous}O_2)}$$

VO_{2max} is limited by both central (cardiovascular and pulmonary) and peripheral (skeletal

muscle) factors, with central factors as most important. It is argued that peripheral factors are

more important in unfit subjects (such as the majority of patients with established CAD)

compared to fit ones[72].

The peak oxygen uptake (VO_{2peak}) is the highest uptake of oxygen obtained during a

cardiopulmonary exercise test, and is often used as a surrogate of VO_{2max}. VO_{2peak} is found to

be the single best predictor of both cardiac and all-cause mortality among patients with

established CVD [68] as well as healthy subjects. This is reflected by the fact that physical

activity is a cornerstone in the secondary prophylaxis of patients with established

atherosclerotic disease. Recommendations on aerobic exercise in CAD patients were revised

and published in a joint statement document in 2012 [73], recommending the following: exercise

3-5 times per week, adequate warm-up of 15 minutes, moderate to high intensity for 20-40

minutes, and cool-down for 5-10 minutes. The recommendations do not distinguish between

SCAD and ACS patients, but calls for care in patients with anterior or apical myocardial infarctions and poor left ventricular function.

Aerobic interval training (AIT) is repeated intervals of exercise with short duration and intensity above the lactate threshold of the subject. After each interval, the subject has an active break, with lower intensity allowing some degree of recovery [74]. Previous studies have demonstrated that AIT is superior to moderate continuous training (MCT) with respect to improving fitness (Figure 6) and endothelial function in patients with CAD [75-78], heart failure [79] and metabolic syndrome [80]. A recent study demonstrated the high safety of both MCT and AIT in patients with CAD [81], arguing in favor of the use AIT in cardiac rehabilitation due to its superior effect on VO_{2peak}.
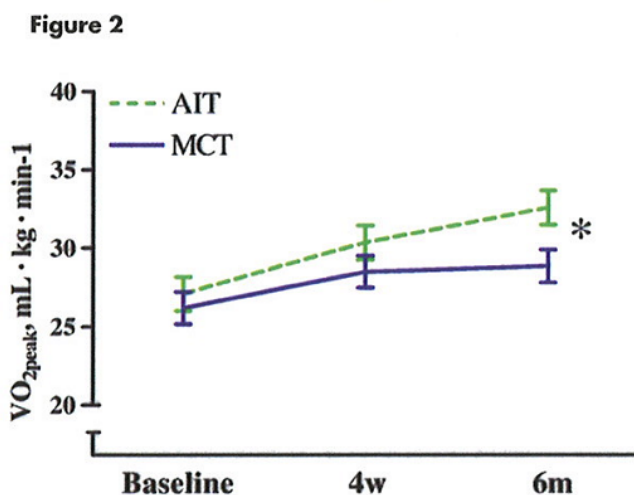
Figure 2

VO_{2peak} in AIT and MCT at baseline, 4w, and 6m. The bars represent ± SEM. *$P < .05$, group comparison.

Figure 6. Superiority of AIT versus MCT in improving VO_{2peak} in patients with CAD. Re-printed with permission from Moholdt et al [75].

1.3.2 The endothelial shear stress hypothesis

Endothelial shear stress can be defined as the stress exerted by the blood flow parallel to the vessel wall (also called laminar shear stress). In healthy subjects, exercise training induces acute alternations in blood flow and endothelial shear stress, the frequency of pulsatile pressure, and systolic blood pressure [82]. Generally, arteries adapt to these forces with structural changes, such as enlargement of the vessel diameter, due to induction of flow-induced vasodilation via a healthy endothelium. This adaption results in normalization of endothelial shear stress, and increased blood flow, but not increased flow velocity [83]. In the coronary circulation, most of the blood flow at rest occurs during diastole. During exercise, coronary blood pressure becomes pulsatile with flow in both systole and diastole, and coronary blood flow is increased five-fold [82]. Endothelial shear stress increases intermittently parallel to increased cardiac output during exercise, and is a potent signal for the integrity and survival of endothelial cells [84], potentially affecting 3 000 endothelial cell genes [85].

The focal distribution of coronary plaques, despite the fact that the entire vasculature is exposed to the same level of systemic risk factors, is attributed to local hemodynamic forces. Endothelial shear stress exerted upon an endothelial cell within the vasculature is a direct function of the vessel geometry in that region. Thus, hemodymanic forces and changes in endothelial shear stress interacting with the endothelium have been implicated in the pathophysiology of CAD [86]. Generally, low endothelial shear stress is considered pro-atherogenic [87] via its effect on coagulation, leukocyte and monocyte migration, smooth muscle growth, lipoprotein uptake and endothelial cell survival [86]. Thus, low endothelial shear stress contributes to the focal distribution of CAD and negative remodeling [88,89]. A central hypothesis in explaining the beneficial effects of exercise in the prevention and treatment of atherosclerotic disease is that exercise produces increased arterial endothelial shear stress, which results in an anti-atherogenic endothelial phenotype [90]. This hypothesis was supported

in a porcine model that demonstrated that low endothelial shear stress promoted the initiation and progression of atherosclerotic plaques [91]. Another study found that coronary segments with high endothelial shear stress at baseline underwent the largest reduction in plaque burden at follow-up [52].

However, some evidence also suggest that high endothelial shear stress may contribute to the development of plaque vulnerability [92], which could be detrimental for patients with established CAD. In a study using RF-IVUS and computational fluid dynamics modeling [93], it was demonstrated that coronary segments with low endothelial shear stress developed greater plaque volume, progression of necrotic core and constrictive remodeling. Coronary segments with high endothelial shear stress developed greater necrotic core and excessive expansive remodeling.

1.3.3 Current evidence for exercise-mediated effects in coronary arteries

Exercise training, as part of a multi-intervention program, has been found to attenuate the progression of coronary atherosclerosis evaluated by coronary angiography [94,95]. It has also been demonstrated that regular exercise slows the progression of coronary plaques in hypercholesterolaemic animal models [96], supporting the hypothesis that exercise induces beneficial changes in coronary atherosclerosis. Improvement of endothelial function, the formation of collaterals and new vessels by vasculogenesis, and reduced arterial stiffness have also been proposed as mechanisms for the positive exercise-mediated effects in patients with established CAD (Table 4) [83,97-101].

There is also evidence for beneficial effects of physical exercise after PCI and stent implantation. In the Exercise Training Intervention after Coronary Angioplasty (ETICA)-trial, patients were randomized to MCT or usual care after coronary angioplasty and/or stent implantation. Improved fitness and a lower extent of restenosis, coronary events and hospital

readmissions were found in the intervention group [102]. In another study it was demonstrated that AIT was associated with a significant reduction in late luminal loss in stented coronary segments evaluated by coronary angiography [103], which suggests that AIT may reduce in-stent restenosis.

Mechanism	Signaling pathway
Improved endothelial function	Increased HDL concentrations
	Increased endothelial nitric oxide synthase
	Decreased generation of reactive oxygen species
	Increased bioavailability of nitric oxide
	Increased amount of endothelial progenitor cells
Vasculogenesis	Decreased generation of reactive oxygen species
	Increased levels of stromal-derived factors and vascular endothelial growth factor
	Mobilization of stem cells
Reduced arterial stiffness	Reduced collagen I and III
	Decreased expression of tumor growth factor beta
	Reduced levels of advanced glycation end-products

Table 4. Proposed signaling pathways for beneficial exercise-mediated effects via increased ESS.

As outlined above, although epidemiological and clinical data support that exercise training has a variety of beneficial effects for both healthy and diseased subjects, there is a paucity of data assessing exercise-induced effects on coronary atherosclerosis, particularly with the use of modern imaging techniques, such as IVUS. To date, no data on plaque composition and exercise in native coronary arteries exist. The main objective of the current thesis was therefore to assess exercise-induced effects on the coronary artery wall and coronary atherosclerosis using IVUS-based imaging modalities.

2 Hypothesis and aims of the thesis

We hypothesized

- That regular aerobic exercise for 12 weeks would induce a general increase in coronary endothelial shear stress and that beneficial changes in coronary plaque geometry and composition would follow.

- That the increase in endothelial shear stress would be larger in subjects exercising with high intensity (AIT) compared to moderate intensity (MCT) and that beneficial changes in coronary plaque geometry and composition would be larger in AIT versus MCT.

- That potential beneficial changes in coronary plaques also depend on clinical factors other than the exercise intervention given.

- That the IVUS inter-pullback reproducibility in stented coronary segments would be poorer than in non-stented segments.

The specific aims of the thesis were

a) To assess the effects of AIT versus MCT on coronary artery plaque geometry in patients with significant CAD on optimal medical treatment using GS-IVUS.

b) To assess the effects of AIT versus MCT on coronary artery plaque composition in patients with significant CAD on optimal medical treatment using RF-IVUS.

c) To assess baseline clinical factors that may be associated with beneficial changes in coronary artery plaque geometry and composition in CAD patients undergoing an aerobic exercise intervention.

d) To compare data from two repeated volumetric GS-IVUS and RF-IVUS pullbacks at a single time-point, thereby assessing inter-pullback variability in different diseased segments in stented coronary arteries.

3 Material and methods

The present thesis is based on a single-center, open, parallel, randomized controlled trial (Paper 1 and 2), and a cross-sectional study (Paper 3). Patients were recruited for the two studies simultaneously. The study protocol was registered at ClinicalTrials.gov (identifier NCT01228201), and performed according to the Helsinki declaration. Written informed consent was obtained from all study participants. Ethical considerations are discussed in section 3.7.

3.1 Patients

Patients were recruited from the Department of Cardiology at St. Olavs Hospital, Trondheim, Norway, from December 2010 to April 2012. Inclusion criteria were age > 18 years and angiographically significant CAD, presented as SCAD or NSTE-ACS, and treated with intracoronary stent implantation. A total of 412 patients were screened for participation before scheduled coronary angiography, and excluded if they met one or more of the following exclusion criteria:

- Inability to perform regular exercise due to comorbidity or work situation
- ST-elevation myocardial infarction
- Non-significant CAD or referral to coronary artery bypass surgery
- Previous surgical revascularization
- Coronary anatomy not suitable for IVUS
- Arrhythmias including atrial fibrillation
- Planned surgery within four months
- Inclusion in another randomized trial

3.2 Study design

All included patients participated in the randomized trial (Paper 1 and 2). Baseline coronary imaging was performed following patient consent (see 3.4), and baseline non-invasive data collection was scheduled 7-10 days after PCI (see 3.3). Following completion of baseline data collection, patients were randomized to AIT or MCT. Randomization was performed by a web-based randomization system developed and administered by Unit of Applied Clinical Research, Institute of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway. Data collection at follow-up was scheduled 2-3 days after completed exercise intervention. A subgroup of patients was randomly picked as participants in the cross-sectional study (Paper 3). In these patients, a second IVUS pullback was performed 5-10 minutes after the first one, either at baseline or at follow-up. Figure 7 shows a flow diagram of enrollment, randomization, follow-up and data analyses in Paper 1, 2 and 3.

In the randomized trial, a difference in cross-sectional plaque area between exercise groups of 40 %, which corresponds to 2.7 mm^3 [104] was estimated. To show this difference with a power of 80 % using a 2-sided test with α=0.05, 19 patients in each group, i.e. 38 patients, were needed [105]. No power calculations were made for the reproducibility study (Paper 3), and it was aimed at including approximately 15 patients based on previous data from the study by Hartmann et al [58].

3.3 Clinical variables and non-invasive data

Information on health, including previous CVDs and medication, was collected from the hospital medical records. Patients were asked about their use of tobacco, categorized as current smoker, previous smoker or never smokers. They were also asked to assess their usual

level of physical activity, classified as inactive if they reported less than 1 hour of hard activity or less than 3 hours of light activity per week, moderately active if they reported 1 to 3 hours of hard activity or above 3 hours of light activity per week, or physically active if they reported more than 3 hours of hard activity per week.
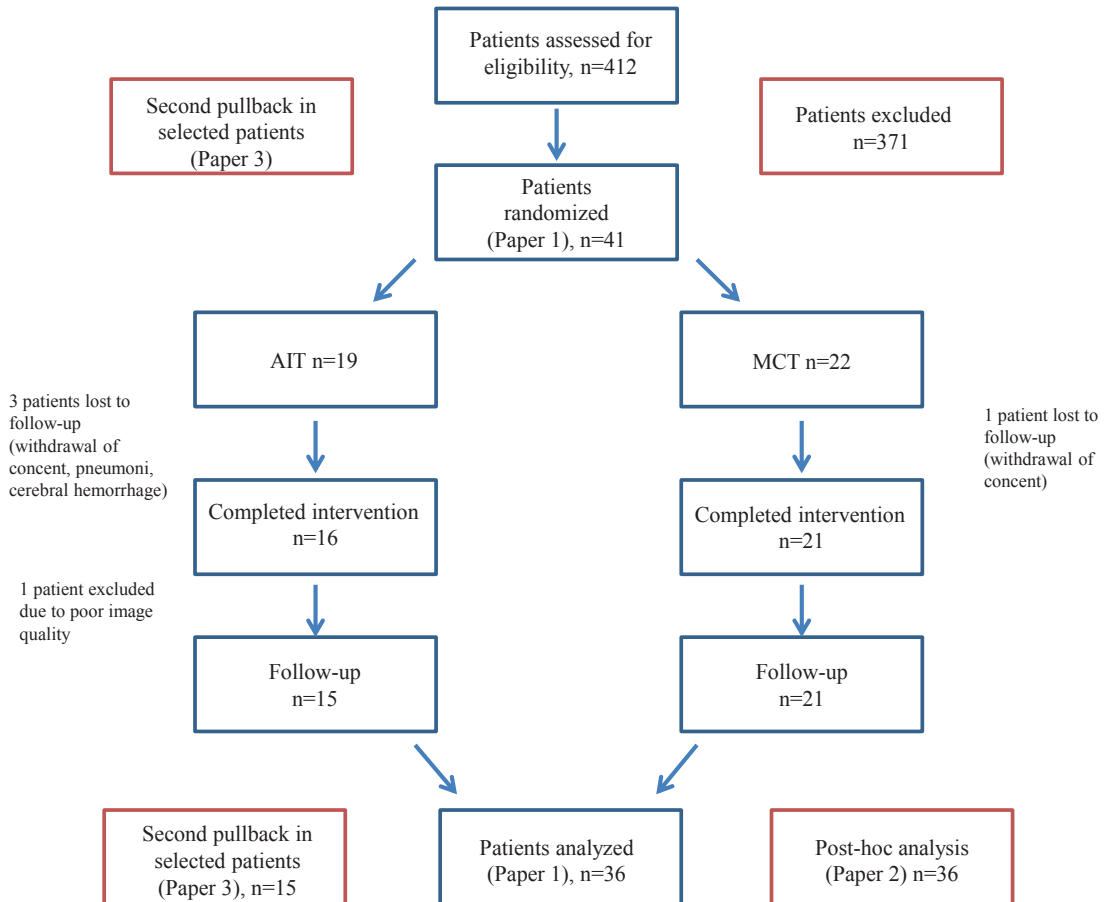
Figure 7. Overview of the studies included in this thesis. See text for details.

Blood pressure was measured using an automatic blood pressure cuff (Welch Allyn, Germany). Height was measured to the nearest cm and body weight was measured to the nearest 0.1 kilogram. Body mass index was calculated as weight divided by the square of height. Waist circumference was measured to the nearest cm at the level of the umbilicus. The heart and lungs were examined with auscultation, and both legs were examined with respect to peripheral edemas at baseline and follow-up. A venous blood sample was drawn between 8.00 am and 9.00 am after over-night fasting. The sample was analyzed for glucose, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, glycosylated hemoglobin, N-terminal pro-brain natriuretic peptide, and C-reactive protein, using accredited in-hospital procedures.

Quality of life was assessed by the Norwegian version of the "MacNew Heart Disease Health Related Quality of Life Questionnaire" (MacNew). The questionnaire consists of 27 items that are organized in three domains; emotional, social and physical, and evaluates how the patient's daily activities and general functioning are affected by the disease and its treatment. The validity and reliability of the Norwegian version of MacNew has been documented [106].

Endothelial-dependent vasodilation was estimated by flow-mediated vasodilation in the brachial artery by a 3-point echocardiography system using a 12-MHz Doppler Probe (Vivid 7 System, GE Vingmed Ultrasound, Horten, Norway). All measurements were performed according to current recommendations [107]. The brachial artery was imaged after ten minutes of supine rest, and continuously for five minutes after cuff deflation. Images were analyzed by an independent observer using automatic edge detection software (Vascular Research Tools 5, Medical Imaging Applications LLC, Coralville, IA, USA). The percentage FMD normalized for shear rate was reported as recommended [108].

3.4 Invasive procedures

Baseline coronary angiography was performed as part of the usual patient diagnostics and treatment. If a patient had signed the consent form, and was diagnosed with at least one epicardial stenosis requiring PCI with stent implantation, the patient was included in the study "on the table". All invasive procedures were performed by very experienced invasive cardiologists and in accordance with current guidelines.

3.4.1 Intravascular ultrasound imaging

Following successful PCI, the index artery was imaged with GS-IVUS and RF-IVUS after intracoronary administration of 200 µg nitroglycerin. This was performed using the Eagle Eye Platinum IVUS 20 MHz probe (Volcano Corporation, Ranco Cordova, CA, USA). A fixed pullback rate of 0.5 mm/s (Volcano R 100 pullback device) was used to standardize volumetric calculations. RF backscattered data were collected with a dedicated console (Volcano Corporation) at every R-peak on the electrocardiogram.

The IVUS probe was advanced as far distally into the index artery as possible, and at least > 10 mm beyond the distal stent edge. Thereafter, the probe was pulled back through the stented segment proximally into the ostium. For participants in the cross-sectional study (Paper 3) this procedure was repeated 5-10 minutes after the first pullback, using the same catheter. Another 200 µg nitroglycerin was administered before the second pullback.

IVUS data were transformed to CDs for later offline analysis. Patients were observed in the ICU after the procedure and were usually discharged from the hospital within 1-3 days depending on clinical presentation and comorbidity. At hospital discharge, a follow-up was scheduled.

3.4.2 Analysis of intravascular data

All intravascular data were analyzed by an independent core laboratory (Krakow
Cardiovascular Research Institute, Krakow, Poland). All analysts were blinded to
randomization group and clinical data. GS-IVUS and RF-IVUS data were analyzed by
automatic contour detection of lumen and vessel by a dedicated program (QIvus software 2.1,
Medis Medical Imaging System, Leiden, the Netherlands). The analyst had to optimize the
automatically drawn contours through image characteristics settings in every single frame. All
analyses were performed according to current recommendations[51,109]. The imaged artery was
divided into five segments of interest (Figure 8);

- *distal reference*: starting as distally as possible to 5 mm from the distal stent edge
- *distal edge*: starting 5 mm distal to the stent edge, ending at the first cross-sectional
  image where the stent covered > 50 % of the vessel wall circumference (defined as
  stent edge)
- *stent*: starting at the first frame after the distal stent edge and ending at the last frame
  before the proximal stent edge
- *proximal edge*: starting at the proximal stent edge and ending 5 mm proximal to the
  stent edge
- *proximal reference*: starting 5 mm proximal to the stent edge and ending as far
  proximally as possible
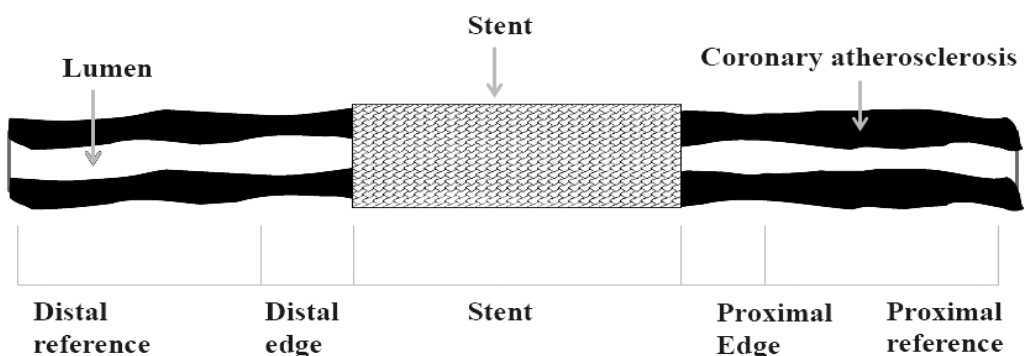
Figure 8. Coronary index artery divided into 5 segments of interest for analysis. Modified from Paper 3 [110].

Matched coronary segments at baseline and follow-up were identified using fiduciary points in the pullback, such as the implanted stent and side braches that were clearly visible in both pullbacks. Parts of segments that were not visible in both pullbacks (baseline and follow-up) were excluded. This was the case in some patients as the catheter was placed more distally in the baseline pullback than in the follow-up pullback, or vice versa. Stented segments were excluded from all RF-IVUS analyses due to the artefacts from stent struts (usually visualized as white and therefore visually interpreted as dense calcium) in RF-analyses.

Computed GS-IVUS parameters in the present thesis are listed and defined in Table 5. Note that the total atheroma volume was normalized for segment length due to large differences in total atheroma volume between patients, thereby providing equal weighting of each patient in the calculations. Normalization was done by dividing the total atheroma volume by segment length and multiplying with the median segment length in the study population.

GS-IVUS parameter	Definition
Minimal lumen area	The smallest cross sectional lumen area detected in the analyzed segment
Total atheroma volume	Cross-sectional area of external elastic membrane minus cross-sectional area of lumen, corresponding to plaque plus media area (Figure 9)
Plaque burden	Plaque plus media area divided by the external elastic membrane area
Remodelling index	Cross-sectional area of external elastic membrane at minimal lumen area divided by the cross-sectional area of external elastic membrane at the smallest plaque burden

Table 5. Analyzed GS-IVUS parameters.

The RF-IVUS parameters were computed based on spectral analysis of the backscattered RF data [111]. Absolute volumes and percentage volumes of the four tissue components fibrous, fibro-fatty, necrotic core, and dense calcium components were analyzed.

Morphological patterns were identified as lesions with a plaque burden > 40 % over 3 consecutive frames separated by 5 mm lengths of artery with a plaque burden < 40 % [51]. Such lesions were classified as separate lesions. These lesions were classified according to the American Heart Association histological classification [112] adapted for RF-IVUS and in terms of vulnerability [51]. Different regions of interest, segments, separate lesions, and study end-points were defined for paper-specific sub-analyses, and are shown in Table 6.
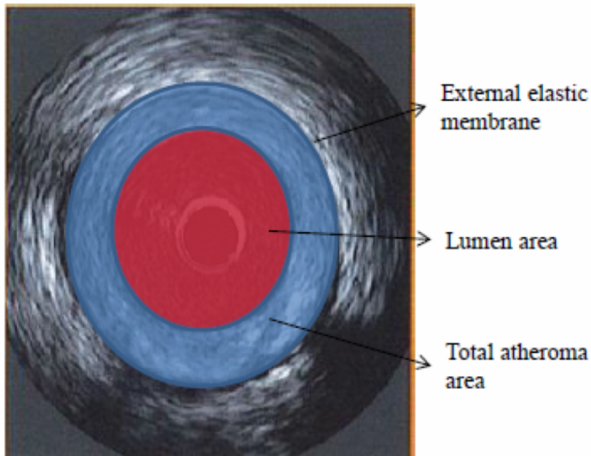
Figure 9. Calculation of total atheroma area (plaque plus media) in a cross-sectional IVUS frame (unit mm^2). The lumen area is subtracted from the external elastic membrane area. Total atheroma volume (unit mm^3) is calculated by software when the segment length is included in the algorithm.

Paper #	Segments of interest	Analytic plan	Study end-points
Paper 1	Proximal and distal reference segments	Whole segments and morphological patterns analyzed	Change in plaque burden and necrotic core. Change in plaque vulnerability for separate lesions
Paper 2	Proximal and distal reference segments	Morphological patterns analyzed	Clinical variables at baseline that were associated with change in plaque burden and necrotic core at follow-up
Paper 3	All five segments	Repeated pullbacks analyzed, only whole segments	Actual and relative difference between pullbacks

Table 6. Segments of interest, analytic plan and study end-points of the papers included in this thesis.

3. 5 Cardiopulmonary exercise testing

Acquisition of non-invasive data other than exercise testing is described in section 3.3. All patients underwent a cardiopulmonary exercise test (repeated at follow-up, Paper 1 and 2) on treadmills (Woodway PPS55, Weil am Rhein, Germany). We used an individualized ramp protocol to last approximately 10 minutes. The majority of patients walked at a predefined speed, and the inclination was raised by 1-3 % every 1-2 minute until complete exhaustion. A few patients ran, and in these patients the inclination was held relatively constant at 2-5 % and the speed was increased every 1-2 minute until complete exhaustion. All patients were monitored with continuous electrocardiogram for safety reasons. Subjective perception of exhaustion was assessed immediately after termination of the test by Borg's 6-20 scale [113].

Gas exchange data were analyzed continuously throughout the test (Oxycon Pro, Jaeger, Hoechberg, Germany). VO_{2peak} was calculated as the mean of the 3 highest measurements during the test. Peak heart rate was defined as the highest heart rate during the test. Heart rate recovery was defined as peak heart rate minus the heart rate after one minute of complete rest standing on the treadmill.

3.6 Aerobic exercise intervention

The exercise protocols were based on protocols used in previous trials in our research group [76-80]. The rationale for using the AIT versus MCT protocols was to introduce isocaloric exercises [78] with different exercise intensity. Both exercise programs consisted of 36 sessions distributed over 12 weeks, i.e. 3 sessions per week. All patients used heart rate monitors (Polar Electro, Kempele, Finland) to help achieve target heart rate during exercise, which was calculated in advance based on the peak heart rate during baseline exercise testing. All sessions were supervised by an experienced physiologist or physician.

The AIT program consisted of 10 minutes of warm-up, followed by intervals of 4 times 4 minutes with an active pause of 3 minutes in-between each interval and at the end. The target heart rate was 85-95 % of peak heart rate during intervals and 60-70 % of peak heart rate during the active pause (Figure 10). Most patients ran during intervals, and walked during the active pause. The MCT program consisted of continuous walking or light running for 46 minutes at ≤ 70 % of peak heart rate. The speed and inclination of the treadmill was individualized for all patients, and recorded together with the target heart rate at 2 minutes and at the end of each interval. An exercise diary was kept for all study participants. Patients in the MCT group were strongly encouraged not to perform exercise with high intensity outside the hospital during the intervention period.
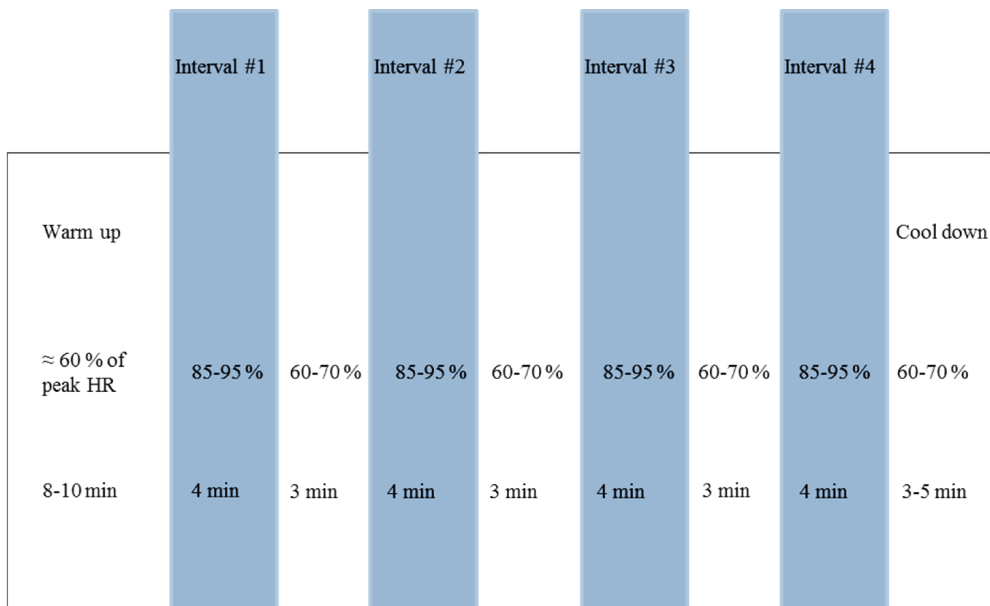
Figure 10. Aerobic interval training model used in Paper I and II. Peak HR = peak heart rate. Modified with permission from Trine Moholdt, Aerobic exercise in coronary heart disease, Doctoral thesis (2010).

3.7 Ethical considerations

The study protocol was approved by the Regional Ethics Committee of Central Norway (identifier 2010/1112). The major issue discussed with the Ethics Committee was the use of invasive imaging in research. However, IVUS is considered safe and the risk for adverse events is low (<0.5 %)[114].

The use of an inactive control group was discussed prior to study registration at clinicaltrials.gov, but this was considered unethical due to the well-documented benefits of exercise in CAD patients [115]. Furthermore, post-PCI patients at our hospital are offered cardiac rehabilitation as part of the routine, and it would have been problematic to deprive patients randomized to an inactive control group from this opportunity.

3.8 Statistical methods

Paper 1 Baseline data were compared between groups using the chi-square test or Mann-Whitney U test. Data measured both at baseline and follow-up were analyzed using repeated measures analysis of variance and linear mixed models with maximum likelihood estimation. Separate lesions in each patient were clustered by patient number where appropriate. The proximal and distal reference segments were analyzed separately. Variables were logarithmically transformed if necessary to achieve adequate model fit.

Paper 2 Statistical analysis was performed as variable screening followed by a final analysis. For the first screening step, the outcome variables were dichotomized into "reduction" versus "no change/increase". Random forest analysis [116,117] (with bootstrapping, n = 2000) was then performed. Baseline explanatory variables that were screened for association with changes in plaque burden or necrotic core included age, sex, body mass index, smoking habits, hypertension, prior myocardial infarction, renal failure, diabetes

mellitus, clinical presentation at baseline (SCAD or NSTE-ACS), cardiovascular medication, blood biomarkers (C-reactive protein, glucose, glycosylated hemoglobin, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides), endothelial function (estimated as percent flow-mediated vasodilation), and peak oxygen uptake. In the second step, significant variables from the random forest analysis were further analyzed using multivariate robust linear regression with the outcome variables in the original continuous form (mm^3). Model fit was evaluated by residual plotting.

Paper 3 The actual difference and relative difference between each parameter in all five artery segments were calculated. The relative difference was normalized for the maximum value for the two measurements. Agreements between pullbacks (i.e. reproducibility) were also assessed visually using Bland-Altman plots by plotting the mean of the two measurements (pullback 1 and 2) on the x-axis and the difference between the two measurements on the y-axis.

Statistical software In Paper 1 and 2 the statistical software SPSS (version 20.0, IBM, Chicago, IL, USA), Stata (version 13.0, StataCorp, College Station, TX, USA), and Minitab (version 16.2.1, Minitab, State College, PA, USA) were used. The statistical package JMP 9.0.0 was used for statistical analysis and data visualization in Paper 3.

4 Summary of results

Forty-one patients were included in the randomized controlled trial (Paper 1 and 2) and 15 patients were included in the cross-sectional study (Paper 3). Drug-eluting stents (Xience Everolimus Eluting Stents, Abbot Vascular, Santa Clara, CA, USA, or Resolute Integrity Zotarolimus Eluting Stents, Medtronic Inc., Minneapolis, MN, USA) were used in 37 patients, whereas four patients received bare-metal stents (Integrity, Medtronic Inc.). The choice of stents was based on clinical considerations by the physician performing stent implantation.

One patient in the AIT group was excluded due to a cerebral hemorrhage, which occurred approximately 36 hours after completion of an exercise session. Otherwise, there were no adverse events in the study, and there were no procedural complications during invasive imaging. Furthermore, three patients did not complete intervention (two patients in the AIT group, and one patient in the MCT group), and data from one patient (AIT group) were not possible to analyze due to very poor imaging quality (Figure 7).

The Core Lab analyzed inter- and intra-observer variability for RF-IVUS analyses. This analysis was performed in 221 regions of interest and showed good correlation between two different analysts (K = 0.79) and excellent correlation for a single analyst (K = 1). Of note, these analyses should not be mixed with the inter-pullback variability, i.e. variability in the acquisition of data itself, which was the study endpoint in Paper 3.

4.1 Paper 1 "Coronary atheroma regression and plaque characteristics assessed by grayscale and radiofrequency intravascular ultrasound after aerobic exercise"

The aim of the study was to assess exercise-induced effects on coronary artery plaque geometry and composition evaluated by GS- and RF-IVUS. The primary end point was

defined as change in plaque burden for the proximal and distal coronary segment and for

separate lesions. The secondary endpoints were defined as change in necrotic core for the

distal and proximal segment and for separate lesions and change in plaque vulnerability for

separate lesions.

Data from 36 patients (7 females) were included. Fifteen patients completed the AIT

protocol, whereas 21 patients completed MCT. All patients completed at least 90 % of

scheduled exercise sessions. Median ages in the two groups were 55.5 and 60.5 years,

respectively. All patients received aspirin, clopidogrel and a statin. Twelve patients in the AIT

group and 7 patients in the MCT group had used statins for > 6 months at study inclusion;

otherwise statins were initiated at admission. VO_{2peak} improved in both groups ($p<0.001$). In

the AIT group VO_{2peak} at baseline was 31.2 (29.1-34.0) $mL*kg^{-1}*min^{-1}$, and at follow-up 34.5

(32.3-37.9) $mL*kg^{-1}*min^{-1}$. The corresponding numbers in the MCT groups were 29.8 (27.5-

33.7) $mL*kg^{-1}*min^{-1}$ and 31.8 (29.1-35.4) $mL*kg^{-1}*min^{-1}$, corresponding to a significant

between-group difference ($p<0.05$) in favor of AIT. A trend for improvement in FMD in both

groups ($p=0.07$) was demonstrated, with no evidence of a difference between groups. All

three quality of life parameters (emotional, social and physical) improved in both groups

($p<0.05$), with no difference between groups.

Twenty coronary segments and 52 separate lesions in the AIT group, and 31 coronary

segments and 92 separate lesions in the MCT groups were analyzed. Necrotic core was

significantly reduced in both groups in distal coronary segments and in separate lesions with

no evidence of a between-group difference. In distal segments, the median reduction was 3.2

% in the AIT group and 2.7 % in the MCT group (both $p<0.05$). In separate lesions, the

median reduction was -2.3 % when analyzing all lesions independently of intervention group

($p<0.05$), and -0.15 mm^3 when lesions were clustered per patient ($p<0.05$). There was a strong

trend toward a reduction in plaque burden independently of intervention group in separate

lesions. The reduction was -10.7 % (-21.7, 0.4, p=0.06). A minority of separate lesions (14.9 %) underwent changes in terms of vulnerability during follow-up (Table 7). In 2 patients in the MCT group we observed that plaques transformed in either direction with respect to vulnerability within the same patient.

Group and patient number	Morphological pattern baseline	Morphological pattern follow-up	Increase or decrease in vulnerability
AIT, patient#1	TCFA/CaTCFA	PIT	Decrease
	TCFA/CaTCFA	PIT	Decrease
	FA/CaFA	FCa	Decrease
AIT, patient#5	FA/CaFA	IMT	Decrease
AIT, patient#17	FCa	PIT	Decrease
AIT, patient#33	TCFA/CaTCFA	IMT	Decrease
MCT, patient#10	FCa	TCFA/CaTCFA	Increase
	FCa	TCFA/CaTCFA	Increase
	TCFA/CaTCFA	IMT	Decrease
MCT, patient#15	TCFA/CaTCFA	IMT	Decrease
	TCFA/CaTCFA	PIT	Decrease
MCT, patient#18	TCFA/CaTCFA	FA/CaFA	Decrease
MCT, patient#21	FCa	TCFA/CaTCFA	Increase
MCT, patient#22	FA/CaFA	PIT	Decrease
	FA/CaFA	PIT	Decrease
MCT, patient#24	PIT	TCFA/CaTCFA	Increase
	IMT	PIT	Increase
MCT, patient#25	PIT	IMT	Decrease
MCT, patient#37	PIT	IMT	Decrease
	IMT	PIT	Increase
MCT, patient#38	PIT	IMT	Decrease
MCT, patient#41	PIT	IMT	Decrease

Table 7. Separate lesions that underwent transformation during the study and were re-classified at follow-up. TCFA/CaTCFA; thin-capped fibroatheroma or calcified thin-capped fibroatheroma, FA/CaFA; fibroatheroma or calcified fibroatheroma, FCa; fibrocalcific, PIT; pathological imtimal thickening, IMT; intimal medial thickening.

In a sensitivity analysis on statin use, we compared changes in plaque burden and necrotic core between patients that used statins at study inclusion and patients that were given statins at baseline. The analysis was performed with and without inclusion of randomization group. There were no significant differences in the reduction of plaque burden and necrotic between the different statin groups (p<0.5 for all tests).

4.2 Paper 2 "Clinical predictors of exercise-induced coronary plaque stabilization: A serial radiofrequency intravascular ultrasound study"

The aim of the study was to assess baseline clinical variables that potentially were associated with an exercise-induced reduction in necrotic core volume or plaque burden in patients with established CAD undergoing PCI with stent implantation. To assess this, data from the randomized controlled trial (Paper 1) was included (n=36). Random forest analysis followed by multivariate robust linear regression was used to identify potential predictors of beneficial exercise-induced changes in coronary plaques.

When analyzing potential predictors of exercise-induced changes in coronary plaques, total cholesterol was the only variable associated with plaque burden reduction at follow-up in the random forest analysis, having a signal that was slightly stronger than random noise. In linear regression, the association between total cholesterol and plaque burden reduction was lost (p=0.33). Significant variables for necrotic core reduction were use of angiotensin enzyme inhibitors or angiotensin II receptor antagonist (weak signal), and clinical presentation (SCAD or NSTE-ACS) at baseline (strong signal). In linear regression, use of angiotensin enzyme inhibitors or angiotensin II receptor antagonist was not significant (p=0.41). Clinical presentation at baseline remained significantly associated with necrotic

core reduction (p=0.011). R-squared for the model including baseline clinical presentation and baseline necrotic core volume was 0.90.

The number of separate lesions per patient, plaque volume at baseline and necrotic core volume at baseline were comparable between the SCAD group and the NSTE-ACS group. The change in plaque volume at follow-up was -6.44 (-24.44;-1.77) mm^3 in SCAD and -1.28 (-14.79;20.50) mm^3 in NSTE-ACS (p=0.19). The change in necrotic core volume at follow-up was -4.94 (-10.33;-1.33) mm^3 in SCAD and 1.03 (-4.29;3.71) mm^3 in NSTE-ACS (p=0.01). The mean necrotic core volume at follow-up was 7.19 (1.87;13.14) mm^3 higher in patients with NSTE-ACS compared to SCAD and necrotic core volume was reduced in 17 patients (94 %) in the SCAD group compared to 8 patients (44 %) in the NSTE-ACS group (p=0.01, Figure 11).
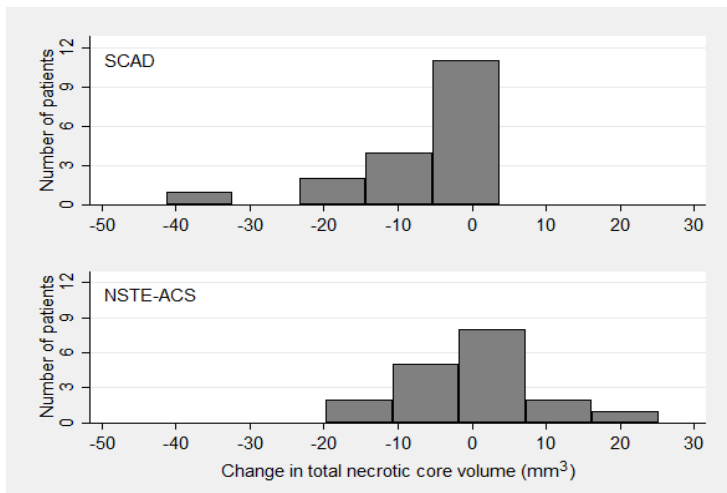
Figure 11. Illustration of change in necrotic core volume at follow-up stratified by clinical presentation.

Statin use prior to study enrollment (> 6 months) and high-density lipoprotein cholesterol levels were the only clinical variables that differed between SCAD and NSTE-

ACS patients at baseline (p<0.05). Fourteen of 18 patients with SCAD used statins prior to study inclusion compared to 5 of 18 patients in the NSTE-ACS group. High-density lipoprotein cholesterol was 1.4 (1.3-1.7) mmol/L and 1.1 (1.0-1.3) mmol/L in the SCAD and NSTE-ACS group, respectively. In a sensitivity analysis, neither statin use prior to study enrollment nor high-density lipoprotein cholesterol were significantly associated with the change in necrotic core volume or plaque burden (p>0.2 for all tests).

4.3 Paper 3 "Reproducibility of grayscale and radiofrequency IVUS data acquisition in stented coronary arteries"

The aim of the study was to assess inter-pullback reproducibility of volumetric GS- and RF-IVUS data in stented coronary arteries. To achieve this, IVUS pullbacks were repeated in the same index artery at a single time-point, and agreements between these two pullbacks were examined.

Fifteen patients (median age 55.2 years, 4 females) were included and examined with 2 separate pullbacks. This resulted in a total of 30 pullbacks and 150 sub-segments for analysis. Inter-pullback reproducibility in GS-IVUS analysis for lumen volumes was very good for all segments with relative differences between pullbacks between ± 5 %. For vessel and plaque volumes, actual differences between pullbacks were low and relative differences were between ± 5 % for all non-stented segments and for the whole vessel. For stented segments, actual differences were somewhat larger, and the relative difference between pullbacks was 5.8 and 7.0 % for the vessel volume and plaque volume, respectively, thus indicating a weaker agreement than for non-stented segments.

In RF-IVUS analysis, inter-pullback reproducibility was generally poorer than in GS-IVUS analysis. For RF-plaque volumes, the relative difference were large for the distal reference segment (-48.2 %) and the distal edge segment (-9.0 %), due to small plaque

volumes in these segments. The actual differences between pullbacks in these segments were comparable to the other segments. The relative difference in RF-plaque volume for the whole non-stented segment was -4.5 % corresponding to very good agreement.

For plaque subtypes volumes, the actual differences were low and the relative differences were -5.3 % for fibrous, -7.0 % for fibro-fatty, 4.8 % for calcium and 2.4 % for necrotic core. Bland Altman plot for RF-plaque volume in the whole vessel is illustrated in Figure 12.
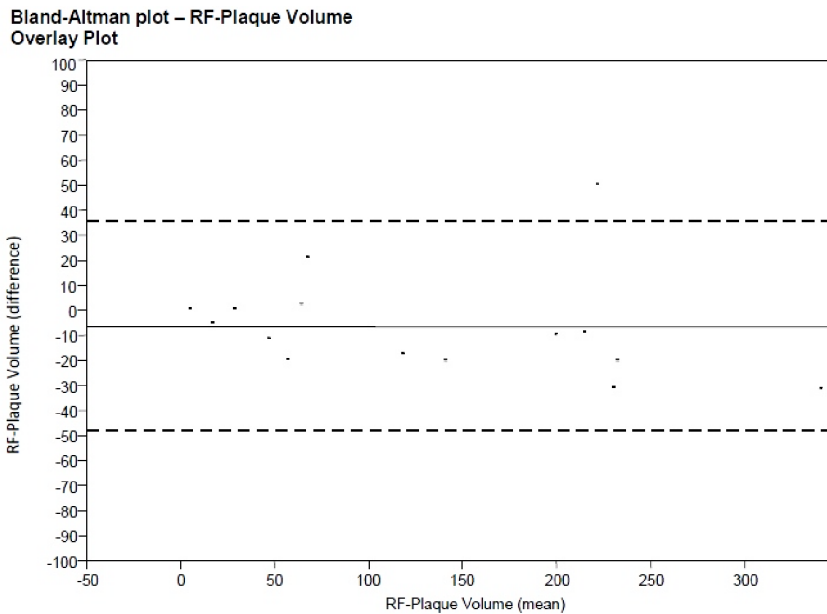
Figure 12. Bland Altman plot displaying the inter-pullback reproducibility between pullbacks for RF-plaque volume in the whole non-stented vessel. The discontinued lines show limits of agreement.

Discussion

The main findings in this thesis were the demonstration of a significant reduction in necrotic core and a strong trend towards a reduction of plaque burden in CAD patients following two different aerobic exercise protocols (Paper 1). These findings support that aerobic exercise has a direct effect on coronary atherosclerosis, not only with respect to atherosclerotic burden, but also with respect to the composition of coronary plaques and potentially also plaque vulnerability.

Further, we found a strong association between the clinical presentation of CAD and necrotic core volume at follow-up. The association was in favor of patients with SCAD compared to NSTE-ACS patients (Paper 2). This finding supports that aerobic exercise may have a particular potential to induce beneficial changes in coronary atherosclerosis in SCAD patients compared to patients in the early phase following an ACS.

Last, we demonstrated that the inter-pullback reproducibility of GS- and RF-IVUS is very good for non-stented coronary segments and acceptable for stented coronary segments. Furthermore, the reproducibility of IVUS parameters in non-stented segments seemed not to be influenced by the stent. These findings may be important for the design of future serial IVUS-studies in intervened vessels.

The thesis presents data from one of the first studies that have assessed exercise-induced effects on the coronary artery wall and coronary atherosclerosis by IVUS-based imaging modalities. To our knowledge, it is the first study in native coronary arteries that has used both GS- and RF-IVUS to assess the effects from aerobic exercise on coronary atherosclerosis, and the first IVUS exercise study that has included ACS patients. Although no randomized trial yet has demonstrated that aerobic exercise improves survival in CAD patients, several surrogate markers of cardiac or vascular function and functional capacity

improve following exercise. This thesis adds knowledge on the physiological adaptations that occur as a result of aerobic exercise in diseased coronary arteries *in vivo*.

Aerobic exercise and atherosclerosis: disease burden versus plaque vulnerability

For obvious reasons, studying coronary atherosclerosis *in vivo* in humans is not easy. Invasive imaging is required to obtain detailed plaque characteristics, and repeated procedures are necessary to assess effects from interventions or to study the natural history of disease. Therefore, animal models have been extensively used in previous research. Studies using hypercholesterolemic mice models have documented slowing of atherosclerosis progression following exercise [96]. Pellegrin et al also found that exercise promoted plaque stabilization [118] via increased fibrous cap thickness and decreased macrophage content in aortic plaques of swim-trained hypertensive Apo E$^{-/-}$ mice [119]. This was demonstrated despite no improvement of cardiovascular risk factors, arguing for a direct effect from exercise on plaque phenotype.

In humans, the easily accessible intima-media thickness measurment of the common carotid artery has been used as a marker of general atherosclerotic burden. Kadoglou et al found an association between physical inactivity and increased intima-media thickness [120], suggesting that physical activity may prevent progression of carotid atherosclerosis. In the coronary circulation, three previous trials have demonstrated a regression of diameter stenosis, evaluated by coronary angiography, in patients undergoing aerobic exercise [94,95,121]. However, these results are limited by the fact that exercise was only one component of the life-style interventions given, and also by the general limitations of coronary angiography to assess atherosclerotic burden, such as underestimation of true disease severity due to diffuse coronary atherosclerosis or coronary remodeling [43].

Gielen et al stated in 2010 that "Surprisingly, not a single study has addressed exercise-mediated changes in plaque volume by intravascular ultrasound thus far to control the findings of the early regression studies with more accurate technique, which also permits insights into changes of plaque composition" [84]. Since then, Sixt et al demonstrated an improvement of coronary endothelial function, but did not find an effect on plaque burden by GS-IVUS, comparing usual care to the combination of 4 weeks in-hospital exercise training with a hypocaloric diet in patients with type 2 diabetes [122]. In heart transplant recipients, Nytroen et al found an attenuated progression of cardiac allograft vasculopathy, but no effect on necrotic core in patients that underwent AIT for one year [123]. Although not directly comparable due to different pathophysiology in native and transplanted coronary arteries, this is in contrast to our findings, demonstrating a significant reduction in necrotic core both in patients undergoing MCT and AIT. The finding of reduced plaque burden as a result of exercise supports the conclusions from previous angiographic studies [94,95,121] and findings from animal exercise models [96,118,119]. The clinical implications of these findings are discussed later on.

Exercise-induced plaque transformation

Our predefined hypotheses stated that regular aerobic exercise would induce a general increase in endothelial shear stress, and that this would induce beneficial changes in coronary plaque geometry and composition. We also hypothesized that the increase in endothelial shear stress would be larger in subjects exercising with high intensity (AIT) compared to moderate intensity (MCT). Our main findings support the first of these hypotheses, but not the second, as the reduction in necrotic core and plaque burden were similar for patients undergoing the two different aerobic exercise protocols. As patients in both groups in our study demonstrated

improved aerobic fitness indicating a significant effect from exercise, it is plausible that this effect could influence coronary plaques through increased endothelial shear stress irrespective of exercise group. However, it needs to be emphasized that endothelial shear stress was not directly measured in our studies.

Our findings of reduced plaque burden following aerobic exercise may support the findings from Koskinas et al, who demonstrated in a swine model that low endothelial shear stress promoted the initiation and progression of atherosclerotic plaques [91]. These findings were also supported by the Prediction of Progression of Coronary Artery Disease and Clinical Outcome Using Vascular Profiling and Shear Stress and Wall Morphology (PREDICTION) study, demonstrating that coronary segments with the highest endothelial shear stress underwent the largest reductions in plaque burden [124].

In terms of plaque vulnerability, previous studies assessing endothelial shear stress and plaque composition have shown conflicting results. Cheng et al used a perivascular shear stress modifier to induce changes in shear stress patterns *in vivo* (lowered, increased and lowered/oscillatory shear stress) in mouse carotid arteries [125]. The main finding from this study was the demonstration of lowered endothelial shear stress inducing larger lesions with increased plaque vulnerability, whereas oscillatory shear stress regions induced stable lesions. A similar model, using computational fluid dynamics, was assessed in humans some years later when Samady et al studied endothelial shear stress in different coronary segments with respect to changes at 6 month follow-up [93]. The investigators of this novel study found that high endothelial shear stress segments developed greater necrotic core and excessive expansive remodeling, suggestive of increased vulnerability, a conclusion in contrast to our hypothesis, and also the findings from Cheng et al [125]. It should be emphasized that exercise-induced changes in endothelial shear stress may have a relatively different effect in rodents than in large mammals [90], including humans, and it is therefore difficult to directly compare

the data from Cheng with our study and the study by Samady. Further exercise studies with measurement or estimation of changes in endothelial shear stress are needed to explore the discrepancies between the conclusions by Samady et al and our study.

We observed that a minority of separate morphological lesions that were identified at baseline underwent transformation at follow-up with respect to plaque vulnerability (Table 7, page 45). Interestingly, we also observed that plaque transformation differed between separate lesions within the same patients. These observations are novel, and support that both systemic and local factors, such as intersegment differences in endothelial shear stress, may be involved in changes of plaque characteristics.

Exercise and coronary atherosclerosis: stable versus unstable disease

The rationale for performing a post-hoc analysis on the data from the randomized controlled trial was the following: AIT and MCT induced similar effects with respect to reduction of plaque burden and necrotic core in our study, and baseline clinical factors that may be associated with changes in these endpoints are unknown.

We found that clinical presentation at study enrollment (i.e. SCAD versus NSTE-ACS) was associated with necrotic core volume at follow-up in favour of patients with stable disease. The R-squared of the model including clinical presentation and baseline necrotic core volume was 0.90, suggesting that these two factors together explain most of the variability in necrotic core volume at follow-up. No other clinical variables were associated with exercise-induced plaque burden reduction. Thus, our results may suggest that aerobic exercise post-PCI has more beneficial effects in patients with SCAD compared to NSTE-ACS.

There are pathophysiological differences between stable and unstable coronary artery disease that could contribute to explaining our findings. Obstructive SCAD is characterized by plaques that have been growing progressively and slowly for many years [38]. The total coronary inflammatory burden is lower than in unstable CAD and the fibrous cap is thick [126]. This condition is rarely fatal, and presents clinically as angina pectoris. After decades of indolent progression of disease however, acute thrombosis leading to acute myocardial ischemia may occur in vulnerable plaques. This will most often present clinically as an ACS or sudden death. Such patients may present with multiple non-culprit vulnerable lesions [127] corresponding to a high inflammatory burden in the coronary circulation. These vulnerable lesions are characterized by multi-focal and focal macrophage densities [128] which are not seen to a similar extent in patients with SCAD.

Maybe even more important, some evidence also suggests that an acute event further accelerates coronary atherosclerosis. Dutta et al demonstrated that Apo $e^{-/-}$ mice developed larger and more advanced plaques following ACS via increased protease activity and monocyte recruitment from the bone marrow [129]. The pro-inflammatory changes in these plaques persisted for several months, corresponding to the intervention period in our study. Although not assessed in humans, this may imply that post-ACS patients suffer from an increased pro-inflammatory load at the same time as these patients usually undergo cardiac rehabilitation.

Based on our findings and the pathophysiological differences between SCAD and NSTE-ACS, it may be hypothesized that an increased pro-inflammatory load rendered patients with unstable disease in our study more resistant to exercise-induced plaque stabilization via decreased necrotic core volume at follow-up. In fact, 10 of 18 patients with NSTE-ACS demonstrated increased necrotic core volume at follow-up. It is also possible that exercise-induced effects on coronary atherosclerosis differ between stable and unstable

patients. Nevertheless, as almost every SCAD patient in our study demonstrated reduced necrotic core volume following aerobic exercise, this intervention may have an underestimated potential for plaque stabilization in stable patients. Whether aerobic exercise, through modification of coronary plaques, translates into reduced risk of coronary events in different coronary populations needs to be explored. It has to be emphasized that our results in Paper II should be considered hypothesis generating leaving more questions than definite answers.

Procedural reproducibility in IVUS imaging

When assessing inter-pullback reproducibility in diseased coronary arteries treated with stent implantation, we found that agreements between pullbacks using GS-IVUS were very good for non-stented segments (relative difference < 5 %), and poorer for stented segments (relative difference < 10 %). Furthermore, agreements between pullbacks using RF-IVUS (excluding the stented segment) were acceptable, but generally poorer than in GS-IVUS analyses. In RF-plaque volume analysis, the relative difference between pullbacks was less than 5 % when analyzing the whole non-stented segment, corresponding to a very good agreement.

These findings add new knowledge regarding reproducibility in stented vessels, and also regarding reproducibility of IVUS parameters in non-stented segments of stented arteries, which seemed not to be influenced by the stent. Our conclusions support the previously reported data from non-intervened vessels. Rodriguez-Granillo et al. demonstrated very reproducible two-dimensional geometrical measurements, and found that RF-IVUS measurements were more variable than GS-variables [130]. These findings were later reproduced by Prasad et al [59], and also by Hartmann et al [58], the latter using volumetric three-dimensional RF-IVUS. All of these studies found that the reproducibility for compositional measurements

(i.e. RF-data) were slightly poorer than for geometrical measurements (i.e. GS-data). The implications of our findings are discussed below.

Clinical implications

Based on our findings in Paper 1, it seems probable that aerobic exercise has a potential to induce beneficial changes in coronary atherosclerosis that no previous intervention has been able to demonstrate by IVUS imaging. In fact, to date, no clinical intervention, including statin therapy[131], has shown a significant reduction of coronary necrotic core volume in serial IVUS studies. Thus, our findings strengthen the scientific evidence for recommending aerobic exercise in CAD patients, and argue for increased use of exercise as medicine.

Furthermore, the beneficial effects of aerobic exercise seem to be larger in patients with SCAD than in patients with NSTE-ACS (Paper 2). Thus, this non-pharmacological and easy accessible intervention may have an underestimated potential for plaque stabilization in the large SCAD population. It is very likely that exercise as effective medicine in this patient group is underused.

It should be emphasized that the studies included in this thesis assessed exercise-induced effects on previously diagnosed coronary atherosclerosis, and not general safety issues with respect to exercise in the general population. We experienced no adverse cardiac events during the study, but the sample size in our study was too small to assess cardiac safety issues with respect to AIT or MCT. However, with respect to CAD patients, a study from our group including over 175 000 training hours has demonstrated that aerobic exercise is safe, also in post-PCI patients undergoing exercise with high intensity [81].

One patient in the AIT group suffered from a cerebral hemorrhage approximately 36 hours after completion of an AIT exercise session. Vigorous exercise has been identified as a possible trigger for rupture of cerebral aneurysms[132]; but the time span between exercise and the clinical event in our study makes increased blood pressure as a result from exercise an unlikely cause of hemorrhage in this particular case. Furthermore, physical activity in general is associated with a decreased risk of cerebrovascular disease [133].

The implications of our study on reproducibility (Paper 3) are probably most relevant to research and in clinical cases where repeated imaging is performed. Based on our data, future serial imaging studies in intervened vessels should account for a variability of 5-10 % attributed to the acquisition of images. Furthermore, the reproducibility of IVUS parameters in non-stented segments of stented coronary arteries seem not to be influenced by the stent itself. These findings may have implications for the design and power calculations in longitudinal studies using IVUS-based endpoints in stented coronary arteries.

Limitations

The use of IVUS-based imaging has obvious strengths such as the ability to visualize cross-sectional images of coronary atherosclerosis. Furthermore, RF-IVUS is one of the few imaging modalities that has the ability to assess tissue components in coronary atheromas *in vivo* [44]. On the other hand, IVUS-based imaging is also hampered with limitations that increase the uncertainty of the method, such as motion artefacts and ultrasound speckle imaging of blood cells in the artery lumen [134]. The IVUS technique also assumes that the vessel is circular and that the catheter is located in the center of the artery with a transducer parallel to the long axis of the vessel. It is possible, however, that both transducer obliquity

and the curvature of the vessel can produce IVUS images that overestimate the dimensions of the vessel [135].

Another important aspect of IVUS imaging is the analysis of data for research use. Dedicated software has been developed to help achieve correct data analysis, but there is still a need for substantial experience when correcting automatically drawn contours in every single frame of the IVUS pullback. Thus, a major strength of the studies included in this thesis is the use of very experienced analysts at an independent Core lab.

The studies included in this thesis are relatively small. However, the study designs with invasive procedures as part of the protocol mean obvious limitations and the number of patients included was equal to or higher than in several other exercise studies[78-80,103,122]. For the randomized controlled trial, no previous exercise trial with IVUS-based endpoints existed when power calculations were made. Thus, our study adds data for power calculations in future exercise studies using serial IVUS imaging.

The most important potential confounder in the randomized controlled trial was statin treatment, which was imbalanced between exercise groups at baseline. It can therefore be argued that this has confounded our results, both with respect to potential differences between groups, but also with respect to the mechanism for the beneficial effects on coronary atherosclerosis that was observed. However, in a sensitivity analysis, there were no differences in the reduction of necrotic core or plaque burden between different statin users, with and without inclusion of exercise group affiliation in the statistical model. Also, low-density lipoprotein cholesterol and C-reactive protein levels were essentially unchanged at follow-up, indicating a modest statin effect during the study. It is therefore unlikely that the differences in statin use between exercise groups at baseline have confounded our results.

Future perspectives

Several aspects of exercise-induced effects on coronary atherosclerosis still remain unknown, and the need for high-quality studies is obvious. In addition to confirming the results from this thesis, new studies need to address both general aspects of exercise as medicine in CAD patients, e.g. exercise modalities for different patient groups, duration of exercise, and the feasibility and adherence to exercise, as well as more specific issues related to exercise-induced mechanisms in the artery wall.

The underlying mechanisms for plaque stabilization and reduced atherosclerotic burden as a result of exercise are still largely unknown. To assess these issues, one has to combine different methodology in well-designed clinical trials and also conduct relevant experimental studies. Furthermore, acknowledging that invasive imaging modalities have different strengths and limitations with respect to assessment of plaque volume and plaque composition, the combination of different imaging modalities such as IVUS, optical coherence tomography (OCT) and near-infrared-spectroscopy (NIRS) should be implemented in invasive study protocols.

Another aspect of exercise in CAD patients is individual responses and also intra-individual responses in the same patient. As hypothesized in Paper 2, it may be that aerobic exercise has different effects in different CAD patient groups. Furthermore, as demonstrated in Paper 1, some patients may experience different plaque effects in different coronary artery segments after an intervention period.

To date, the concept of personalized medicine has to a little degree referred to the use of exercise as therapeutic medicine. Therefore, more knowledge of different responses in different patient groups, including clinical diagnosis, age, gender and co-morbidities, are needed.

An important factor is to motivate people to be more active in everyday life. Today, clinicians experience substantiated problems with patient adherence to exercise recommendations, and an increasing social gradient with respect to CVD risk factors has to be acknowledged. In many cases, a large number of practical obstacles need to be sorted out before patients start to exercise, and the many barriers to make patients to stay physically active in the long run are even more problematic. These challenges should also be addressed in future studies.

6   Conclusions

a) In patients with significant CAD treated with stent implantation and optimal medical treatment, AIT or MCT for 12 weeks induced a strong trend towards a plaque burden reduction in separate morphological coronary lesions assessed by GS-IVUS.

b) Using RF-IVUS, aerobic exercise induced a reduction in coronary necrotic core, both in distal coronary segments and in separate morphological lesions, by approximately 3 % and 0.15 mm^3, respectively.

c) Reduction of coronary necrotic core volume in patients undergoing aerobic exercise was much more frequent in patients with SCAD than NSTE-ACS, and may be strongly dependent on the clinical and pathophysiological nature of coronary atherosclerosis.

d) The inter-pullback reproducibility in stented coronary arteries was very good for GS-data in non-stented segments with relative differences between pullbacks of < 5 %. The inter-pullback reproducibility was poorer for RF-data and GS-data in stented segments, but still acceptable with relative differences between pullbacks of < 10 %.

7 References

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Paper I

Coronary Atheroma Regression and Plaque Characteristics Assessed by Grayscale and Radiofrequency Intravascular Ultrasound After Aerobic Exercise

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The aim of the present study was to investigate effects of aerobic interval training (AIT) versus moderate continuous training (MCT) on coronary atherosclerosis in patients with significant coronary artery disease on optimal medical treatment. Thirty-six patients were randomized to AIT (intervals at ≈ 90% of peak heart rate) or MCT (continuous exercise at ≈ 70% of peak heart rate) 3 times a week for 12 weeks after intracoronary stent implantation. Grayscale and radiofrequency intravascular ultrasounds (IVUS) were performed at baseline and follow-up. The primary end point was the change in plaque burden, and the secondary end points were change in necrotic core and plaque vulnerability. Separate lesions were classified using radiofrequency IVUS criteria. We demonstrated that necrotic core was reduced in both groups in defined coronary segments (AIT −3.2%, MCT −2.7%, p <0.05) and in separate lesions (median change −2.3% and −0.15 mm^3, p <0.05). Plaque burden was reduced by 10.7% in separate lesions independent of intervention group (p = 0.06). No significant differences in IVUS parameters were found between exercise groups. A minority of separate lesions were transformed in terms of plaque vulnerability during follow-up with large individual differences between and within patients. In conclusion, changes in coronary artery plaque structure or morphology did not differ between patients who underwent AIT or MCT. The combination of regular aerobic exercise and optimal medical treatment for 12 weeks induced a moderate regression of necrotic core and plaque burden in IVUS-defined coronary lesions. © 2014 Elsevier Inc. All rights reserved. (Am J Cardiol 2014;114:1504−1511)

Physical activity is associated with reduced mortality risk in patients with coronary artery disease (CAD).[1] Aerobic capacity is also found to be a strong predictor of mortality in patients with CAD.[2] The mechanisms responsible for the favorable effects of exercise are not completely understood and may include both modification of cardiovascular risk factors[3] and a direct effect on atherosclerosis.[4] It is hypothesized that increased endothelial shear stress (ESS) produced by bouts of exercise acts antiatherogenically through the modulation of endothelial cell phenotypes.[5] However, high ESS produced by exercise may also increase vulnerability in established plaques,[6] possibly leading to coronary events.[7]

Three previous trials have documented favorable effects of exercise on CAD evaluated by coronary angiography.[8−10] However, there is a paucity of data demonstrating effects of exercise on coronary atherosclerosis evaluated by intravascular ultrasound (IVUS).[11] In the present study, we assessed the effects of 2 different aerobic exercise protocols on coronary plaque structure and morphology in patients with CAD using grayscale intravascular ultrasound (GS-IVUS) and radiofrequency intravascular ultrasound (RF-IVUS).

Methods

This was a single-center, open, parallel, randomized controlled trial. The study protocol was approved by the Regional Ethics Committee of Central Norway (2010/1112), registered at the *ClinicalTrials.gov* (identifier NCT01228201) and performed according to the Declaration of Helsinki. Written informed consent was obtained from all participants. Forty-one patients with angina pectoris or non-ST elevation acute coronary syndrome treated with stent implantation were found eligible and enrolled in the study from December 2010 to April 2012. An overview of the study, including the exclusion criteria, is presented in Figure 1. All patients received standard in-hospital care and optimal medical treatment according to the current guidelines. After baseline data acquisition, patients were randomized to aerobic interval training (AIT, 19 patients) or moderate continuous training (MCT, 22 patients) by block randomization using a
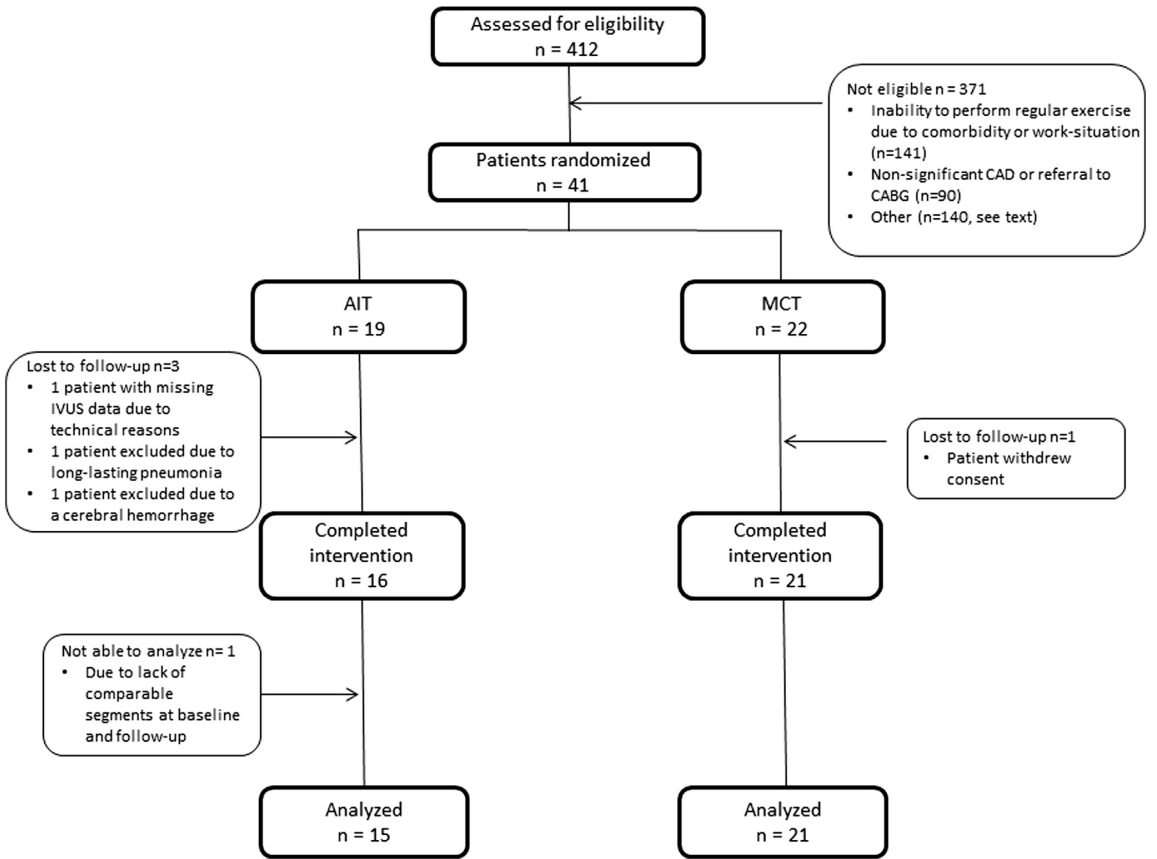
Figure 1. Flow diagram of enrollment, randomization, follow-up, and data analyses. Other: Previous surgical revascularization (n = 53), arrhythmias including atrial fibrillation (n = 40), lack of consent to participate (n = 14), coronary artery anatomy not suitable for IVUS (n = 13), planned surgery within 4 months (n = 11), or inclusion in another randomized trial (n = 9). CABG = coronary artery bypass grafting.
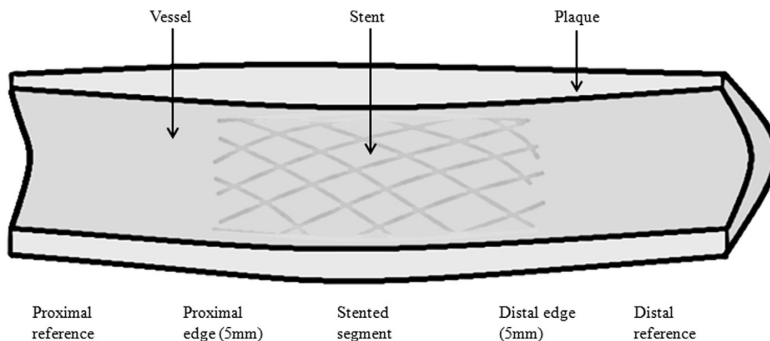
Figure 2. Schematic drawing of analyzed coronary artery segments. Data from the proximal and distal reference segments were included in the present study.

Web-based randomization tool. Data acquisition was repeated at follow-up 2 to 3 days after completed intervention.

An individually adjusted cardiopulmonary exercise test was performed on treadmills (Woodway PPS55, Weil am Rhein, Germany), and gas exchange data were analyzed (Oxycon Pro; Jaeger, Hoechberg, Germany). Peak oxygen uptake (VO_{2peak}) was calculated as the mean of the 3 highest VO_2 measurements during the test. The highest heart rate (HR) during the test was recorded as peak HR, and HR recovery was defined as the change in peak HR to HR after 1 minute of rest. Subjective perception of exertion was assessed using the Borg 6-20 scale.

Table 1
Baseline characteristics of the study population

Variable	Aerobic Interval Training (n = 15)	Moderate Continuous Exercise (n = 21)
Age (years)	55.5 (50−60.5)	60.5 (56.5−63.5)
Male/female	14/1	15/6
Current smoker	3 (20%)	3 (14%)
Hypertension	8 (53%)	12 (57%)
Diabetes mellitus	5 (33%)	3 (14%)
Hypercholesterolemia	4 (27%)	7 (33%)
Prior myocardial infarction	1 (7%)	4 (19%)
Prior percutaneous coronary intervention	3 (20%)	5 (24%)
Current diagnosis		
Angina pectoris	7 (47%)	11 (52%)
NSTE-ACS	8 (53%)	10 (48%)
No. of coronary arteries narrowed		
1	11 (73%)	13 (62%)
2	3 (20%)	8 (38%)
3	1 (7%)	0 (0%)
Target coronary artery location		
Left ascending	9 (60%)	10 (48%)
Circumflex	2 (13%)	4 (19%)
Right	4 (27%)	7 (33%)
Segments assessed		
Distal to the stent	11	17
Proximal to the stent	9	14
Segment lengths (mm)		
Distal to the stent	14.1 (7.1−22.0)	25.0 (19.2−31.6)*
Proximal to the stent	13.5 (7.5−23.0)	15.2 (9.8−20.7)
Identified separate lesions	52 (36%)	92 (64%)
Medication		
Aspirin	15 (100%)	21 (100%)
Clopidogrel	15 (100%)	21 (100%)
Statins	15 (100%)	21 (100%)
Simvastatin 40 mg	12 (80%)	15 (71%)
Atorvastatin 40 mg	3 (20%)	6 (29%)
Beta blockers	12 (80%)	15 (71%)
ACE enzyme inhibitors or angiotensin II receptor antagonists	8 (53%)	11 (52%)

Note: Data are given as numbers with percentages or medians with 95% confidence intervals in parenthesis.

* Significant difference between groups (p <0.05).

All patients exercised on treadmills 3 times a week for 12 weeks with attendance ≥ 90% of sessions in all patients. Training sessions were supervised by experienced staff, and patients used HR monitors (Polar Electro, Kempele, Finland) to help achieve target HR during exercise. The AIT program was based on previous protocols from our research group[12−14] and consisted of 10 minutes of warm-up followed by intervals of 4 times 4 minutes, with an active pause of 3-minute in-between intervals and at the end. The target HR was 85% to 95% of the peak HR during intervals and 70% of peak HR in the active pause. The MCT program was isocaloric to the AIT program[15] and consisted of continuous walking or light running for 46 minutes at 70% of maximum HR.

Quality of life parameters were assessed by the MacNew Heart Disease Health-Related Quality of Life Questionnaire.

A fasting venous blood sample was analyzed for glucose, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, glycosylated hemoglobin, N-terminal pro-brain natriuretic peptide, and C-reactive protein. Endothelial-dependent vasodilation in the brachial artery was estimated by flow-mediated vasodilation (FMD) according to guidelines[16] using a 12-MHz Doppler Probe (Vivid 7 System; GE Vingmed Ultrasound, Horten, Norway). Images were analyzed by an independent observer using an automatic edge detection software (Vascular Research Tools 5; Medical Imaging Applications LLC, Coralville, IA). The percentage FMD normalized for shear rate was reported.

Coronary angiography and percutaneous coronary intervention with stent implantation were performed in accordance with international guidelines. Drug-eluting stents (Xience Everolimus Eluting Stents, Abbot Vascular, Santa Clara, CA or Resolute Integrity Zotarolimus Eluting Stents, Medtronic Inc, Minneapolis, MN) were used in all patients except 4 (1 in the AIT group and 3 in the MCT group) who received bare metal stents (Integrity, Medtronic Inc). GS- and RF-IVUS were performed without complications, after intracoronary administration of 200 μg of nitroglycerin, using the Eagle Eye Platinum IVUS 20 MHz probe (Volcano Corporation, Rancho Cordova, CA) with a pullback rate of 0.5 mm/s (Volcano R100 pullback device). RF backscatter data were collected with a dedicated console (Volcano Corporation) at every R-peak on the electrocardiogram. The IVUS probe was advanced as far distally as possible and >10 mm beyond the distal stent edge, and pullbacks were continued through the stented region and proximally into the ostium of the index artery. Data were stored digitally for analyses at an independent core laboratory (Krakow Cardiovascular Research Institute, Krakow, Poland). All analysts were blinded to clinical data and randomization group.

Intravascular data were analyzed (QIvus software 2.1; Medis Medical Imaging Systems, Leiden, The Netherlands) by automatic contour detection of lumen and vessel according to current recommendations.[17,18] The analyst optimized contours through image characteristic settings and compared the correctness of each edge of automatically drawn contours. Matched coronary segments at baseline and follow-up were identified using fiduciary points, such as side braches and the implanted stent. Parts of segments that were not visible in both pullbacks were excluded. Two regions of interest were defined in the analytic plan: the distal segment, starting distally as far as possible to 5 mm from the distal stent edge, and the proximal segment, starting 5 mm from the proximal stent edge to as far proximally as possible (Figure 2). The following GS-IVUS parameters were computed: minimal lumen area, plaque burden (plaque plus media area divided by the vessel area), total atheroma volume (cross-sectional area of external elastic membrane minus cross-sectional area of lumen) normalized for segment length, and remodeling index (cross-sectional area of external elastic membrane at minimal lumen area divided by the cross-sectional area of external elastic membrane at the smallest plaque burden). The RF-IVUS parameters were computed based on spectral analysis of the backscattered RF data. Absolute volumes and percentage volumes of fibrous, fibro-fatty, necrotic core, and dense calcium components were analyzed (data not shown for other tissue components than necrotic core).

Table 2

Anthropometric data, biomarkers, and flow-mediated vasodilation at baseline and follow-up

	Aerobic Interval Training		Moderate Continuous Exercise	
	Baseline	Follow-Up	Baseline	Follow-Up
Body mass index (kg/m^2)	27.3 (25.3−29.4)	27.4 (25.6−29.2)	26.3 (24.7−28.3)	26.3 (24.5−28.3)
Waist circumference (cm)	100 (93−104)	100 (92−102)	100 (94−104)	97 (92−102)
C-reactive protein (mg/L)	3.65 (1.55−5.93)	2.30 (1.35−3.63)	2.57 (1.59−3.75)	1.93 (1.17−3.9)
N-terminal pro-brain natriuretic peptide (ng/L)	65 (45−161)	67 (28−199)	189 (104−372)	118 (75−207)
Glucose (mmol/L)	5.8 (5.5−7.2)	6.0 (5.5−6.9)	5.8 (5.5−6.2)	5.8 (5.5−6.3)
Glycosylated hemoglobin (%)	5.8 (5.5−7.1)	5.8 (5.5−6.5)	5.9 (5.7−6.2)	5.8 (5.6−6.1)
Total cholesterol (mmol/L)	4.3 (3.8−4.6)	4.2 (3.7−4.7)	4.4 (4.2−4.6)	4.4 (4.2−4.7)
LDL-cholesterol (mmol/L)	2.4 (2.2−2.7)	2.2 (2.0−2.6)	2.4 (2.2−2.7)	2.3 (2.2−2.5)
HDL-cholesterol (mmol/L)	1.2 (1.0−1.4)	1.2 (1.1−1.4)	1.3 (1.1−1.5)	1.4 (1.2−1.6)
Triglycerides (mmol/L)	1.1 (0.9−1.9)	1.2 (1.0−1.7)	1.2 (1.0−1.5)	1.3 (1.0−1.8)
Flow-mediated vasodilation (%)	8.4 (5.2−12.1)	11.3 (7.3−16.7)	11.9 (7.2−17.0)	15.1 (9.8−24.6)

Note: Data are given as medians with 95% confidence intervals in parenthesis. No significant differences within or between groups.

Table 3

Cardiorespiratory variables at baseline and follow-up

	Aerobic Interval Training		Moderate Continuous Training	
	Baseline	Follow-Up	Baseline	Follow-Up
Resting heart rate (beats/min)	61 (56−65)	58 (52−63)	58 (54−63)	59 (55−63)
Peak heart rate (beat/min)	158 (150−167)	162 (154−169)	155 (146−164)	158 (148−168)
Heart rate recovery, 1 minute	29 (24−33)	30 (26−36)	27 (24−31)	27 (24−31)
Peak oxygen uptake (mL*kg^{-1}*min^{-1})	31.2 (29.1−34)	34.5 (32.3−37.9)*†	29.8 (27.5−33.7)	31.8 (29.1−35.4)*
Peak oxygen uptake (mL*min^{-1})	2750 (2423−2954)	3084 (2730−3385)*†	2545 (2146−2888)	2722 (2307−3101)*
Respiratory exchange ratio at peak oxygen uptake	1.12 (1.09−1.14)	1.12 (1.10−1.15)	1.11 (1.09−1.14)	1.11 (1.09−1.13)
Borg scale at peak oxygen uptake	17 (16−17)	18 (17−18)	17 (16−18)	18 (17−18)

Note: Data are given as medians with 95% confidence intervals in parenthesis.
* Significant within-group difference from baseline to follow-up (p <0.05).
† Significant between-group difference from baseline to follow-up (p <0.05).

Table 4

Grayscale and radiofrequency intravascular ultrasound data at baseline and follow-up in segments proximal and distal to the implanted stent

	Aerobic Interval Training		Moderate Continuous Training	
	Baseline	Follow-Up	Baseline	Follow-Up
Proximal segments				
Minimal lumen area (mm^2)	6.07 (5.25−8.13)	5.99 (4.90−7.16)	6.08 (4.59−7.92)	5.82 (4.20−7.49)
Total atheroma volume† (mm^3)	9.10 (5.40−22.60)	8.30 (5.30−26.10)	10.70 (6.10−37.50)	10.10 (6.20−39.8)
Plaque burden (%)	50.7 (42.8−59.0)	51.9 (40.5−59.8)	48.8 (39.5−55.3)	48.3 (42.0−56.6)
Remodeling index	0.97 (0.86−1.18)	1.02 (0.95−1.16)	0.94 (0.87−1.01)	0.93 (0.86−0.99)
Necrotic core volume (mm^3)	24.6 (9.3−42.8)	23.3 (7.0−40.4)	16.5 (4.2−27.0)	13.7 (6.2−42.8)
Necrotic core volume (%)	22.6 (17.3−29.2)	20.9 (15.2−30.8)	18.4 (14.8−22.0)	18.5 (13.7−22.6)
Distal segments				
Minimal lumen area (mm^2)	5.14 (4.24−6.75)	4.78 (4.10−8.07)	4.77 (3.95−5.58)	5.24 (4.28−6.14)
Total atheroma volume† (mm^3)	3.96 (1.95−8.06)	3.70 (1.69−7.43)	6.96 (4.08−10.32)	6.53 (3.93−10.42)
Plaque burden (%)	30.7 (23.5−38.7)	28.4 (22.8−38.2)	40.4 (33.9−46.8)	38.4 (32.6−45.1)
Remodeling index	0.88 (0.78−0.98)	0.95 (0.83−1.10)	0.94 (0.86−1.02)	0.98 (0.90−1.04)
Necrotic core volume (mm^3)	3.2 (0.1−12.5)	2.1 (0−11.6)	18.4 (5.0−31.6)	10.7 (3.6−23.6)
Necrotic core volume (%)	17.9 (9.6−23.7)	14.7* (6.2−20.7)	18.6 (14.0−23.2)	15.9* (11.7−19.2)

Note: Data are given as medians with 95% confidence intervals in parenthesis.
* Significant within-group difference from baseline to follow-up (p <0.05).
† Normalized for segment length.

Morphologic patterns were classified[18] by identifying plaques with a plaque burden >40% over 3 consecutive frames separated by 5 mm lengths of artery with a plaque burden <40% (defined as separate lesions). Separate lesions were classified according to the American Heart Association histological classification[19] adapted for RF-IVUS and in
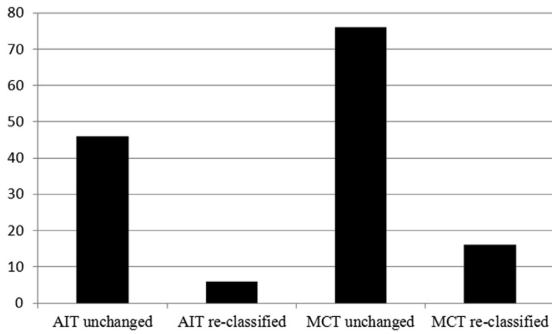
Figure 3. Classification of separate lesions at follow-up stratified by exercise group. Axis shows number of lesions.
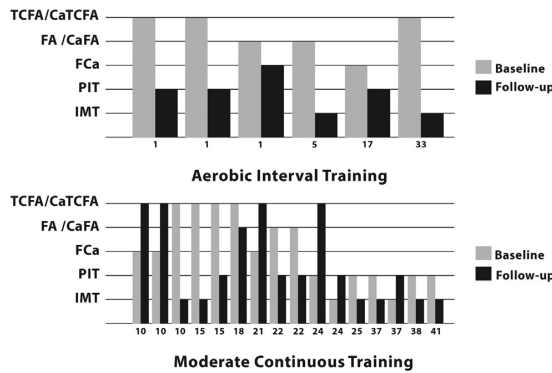

Figure 4. Separate lesions that underwent transformation during the study and that were reclassified at follow-up (14.9% of all separate lesions). Vertical axis shows lesion type with increased vulnerability along the axis, and horizontal axis shows patient study number. FA/CaFA = fibroatheroma or calcified fibroatheroma; FCa = fibrocalcific; IMT = intimal medial thickening; PIT = pathologic intimal thickening; TCFA/CaTCFA = thin-capped fibroatheroma or calcified thin-capped fibroatheroma.

terms of vulnerability[18] in the following order: intimal medial thickening, pathologic intimal thickening, fibrotic or fibrocalcific, fibroatheroma or calcified fibroatheroma, and thin-capped fibroatheroma (TCFA) or calcified thin-capped fibroatheroma (CaTCFA), with TCFA or CaTCFA being highest risk plaques. Inter- and intraobserver variability for RF-IVUS analyses (performed on 221 regions of interest) showed good to excellent correlation between 2 different analysts and for a single analyst ($\kappa = 0.79$ and $\kappa = 1$, respectively).

The primary end point was defined as change in plaque burden for the proximal and distal coronary segment and for separate lesions. The secondary end points were defined as change in necrotic core for the proximal and distal coronary segment and for separate lesions and change in plaque vulnerability for separate lesions. Data were analyzed using SPSS (version 20.0; IBM, Chicago, IL), Stata (version 12.1; StataCorp, College Station, TX), and Minitab (version 16.2.1; Minitab, State College, PA). Data are given as frequencies and percentages or medians with 95% confidence intervals in parenthesis because many variables were not normally distributed. Baseline characteristics were compared using the chi-square test or Mann-Whitney U test. Data measured twice were analyzed using repeated measures analysis of variance and linear mixed models with maximum likelihood estimation. Where appropriate, plaques in each patient were clustered by patient number. Variables were logarithmically transformed if necessary to achieve adequate model fit. p values <0.05 were considered statistically significant.

Results

One patient in the AIT group was excluded because of a cerebral hemorrhage. Otherwise, there were no adverse events in the study. Furthermore, 3 patients did not complete intervention, and data from 1 patient were not possible to analyze (Figure 1).

Baseline clinical characteristics are given in Table 1. The distal segment length was longer in the MCT group (p <0.05), and therefore, total atheroma volume was normalized for segment length in the analyses as recommended.[17] Twelve patients in the AIT group and 7 patients in the MCT group had used statins for >6 months before study inclusion (difference between groups, p <0.05). Otherwise, statins were initiated at the time of admission ± 1 day. To account for potential confounding from differences in statin use, changes in plaque burden and necrotic core were calculated between the different categories of statin users, with and without inclusion of exercise group affiliation. There were no differences in the reduction of necrotic core or plaque burden between the different statin groups in any analyses (data not shown, p >0.5 for all tests).

Table 2 presents anthropometric data, biomarkers, and FMD. There was a trend for improvement in FMD in both groups (p = 0.07), with no difference between groups. Quality of life parameters (emotional, physical, and social domains) improved in both groups (p <0.05), with no difference between groups (data not shown). VO_{2peak} improved in both groups (p <0.001), and the improvement was larger in the AIT group (p <0.05, Table 3).

Twenty coronary segments and 52 separate lesions in the AIT group and 31 coronary segments and 92 separate lesions in the MCT group were analyzed (Table 4). Necrotic core was significantly reduced, independent of intervention group, both in separate lesions and in distal coronary segments. In separate lesions, the median reduction was -2.3% (-4.4, -0.3) when analyzing all lesions (p <0.05) and -0.15 mm^3 (-0.04, -0.27) when lesions were clustered per patient (p <0.05). There was a strong trend toward a reduction in plaque burden of -10.7% (-21.7, 0.4) independent of intervention group in separate lesions (p = 0.06).

Separate lesions at baseline (AIT/MCT, respectively) were classified as 16 of 21 intimal medial thickenings, 1 of 14 pathologic intimal thickening, 5 of 6 fibrocalcifics, 6 of 10 FAs/CaFAs, and 24 of 41 TCFAs/CaTCFAs. A minority of lesions (14.9%) changed in terms of plaque vulnerability during follow-up (Figure 3). All 6 lesions in the AIT group that were reclassified were transformed into less vulnerable lesions (Figures 4 and 5). In the MCT group, 10 lesions were transformed into less vulnerable lesions (Figure 5), and 6 lesions were transformed into more vulnerable lesions. In
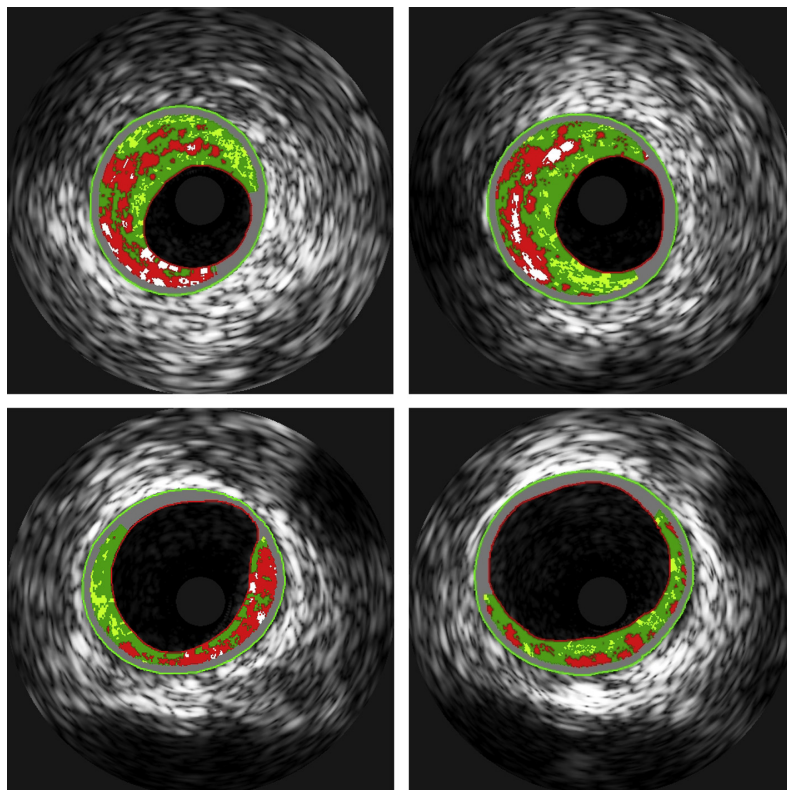
Figure 5. Radiofrequency IVUS cross-sectional images from 2 patients (above, moderate continuous training; below, aerobic interval training) at baseline and follow-up illustrating transformation into less vulnerable lesions. Separate lesions underwent transformation from a thin-capped fibroatheroma (above, *left*) to a fibroatheroma (above, *right*) and from a thin-capped fibroatheroma (below, *left*) to intimal medial thickening (below, *right*). Green = fibrous tissue; green-yellowish = fibro-fatty tissue; red = necrotic core; and white = dense calcium.

3 patients in the MCT group (patient numbers 10, 24, and 37, Figure 4), we observed that plaques transformed in either direction with respect to vulnerability within the same patient.

Discussion

We assessed the effects of 2 different aerobic exercise programs on coronary atherosclerosis evaluated by GS- and RF-IVUS. Changes in coronary artery plaque structure and morphology did not differ between patients who underwent AIT or MCT. The combination of regular aerobic exercise and optimal medical treatment for 12 weeks induced a moderate regression of plaque burden and necrotic core in IVUS-defined coronary lesions.

More than 30 years ago, it was demonstrated that exercise prevented CAD in monkeys that were on an atherogenic diet.[20] Since then, other animal studies have also supported that exercise has antiatherogenic effects.[21,22] However, direct evidence of such effects in humans is difficult to obtain. In 3 previous trials, a regression of diameter stenosis evaluated by coronary angiography was found in patients who underwent exercise training.[8–10] To our knowledge, only 1 previous study has evaluated exercise-induced effects on native CAD with IVUS in humans. In this study, using a multifactorial intervention program and not including assessment of plaque composition, there was no evidence of reduced plaque burden in the intervention group.[11]

We found a strong trend toward a 10% reduction in plaque burden in IVUS-defined separate lesions with no evidence of a difference between exercise groups, despite a larger increase in VO_{2peak} in the AIT group. A possible explanation for this observation could be that the introduction of regular exercise in most patients represented a major intervention and change in lifestyle irrespective of exercise protocol. One may speculate that a prolonged intervention period could have induced larger reductions in plaque burden and revealed differences between groups.

It is hypothesized that increased ESS, induced by increased coronary blood flow produced by bouts of exercise, is responsible for an antiatherogenic effect through alternations of multiple gene expression in endothelial cells.[5] Koskinas et al[23] demonstrated in swine that low ESS promoted the initiation and progression of plaques. This was supported by the Prediction of Progression of Coronary Artery Disease and Clinical Outcome Using Vascular Profiling of Shear Stress and Wall Morphology study, demonstrating that high ESS coronary segments underwent the largest

reduction in plaque burden.[24] In contrast, high ESS segments developed greater necrotic core and excessive expansive remodeling, suggestive of increased vulnerability, in a study by Samady et al,[6] using computational fluid dynamics.

In the present study, we hypothesized that regular aerobic exercise would induce a general increase in coronary artery ESS and possibly more so in patients who underwent AIT. Both groups in our study demonstrated improved physical capacity at follow-up indicating a significant effect from exercise that theoretically could influence plaque composition through increased ESS irrespective of exercise group. The finding of reduced necrotic core in our study is, therefore, in contrast to the data from Samady et al.[6] Even though a general boost of increased ESS during exercise can be anticipated, the influence of exercise on coronary plaques could differ substantially between coronary segments. We observed that plaque transformation differed between separate lesions in different coronary segments within the same patients (Figure 4) supporting that both universal and local factors, such as intersegment differences in ESS, may be involved in changes of plaque characteristics.[23] It is also possible that the mechanisms for the necrotic core reduction as demonstrated in our study could be other than increased ESS.

The Providing Regional Observations to Study Predictors of Events in the Coronary Tree study demonstrated that nonculprit lesions being responsible for unanticipated coronary events were characterized by a large plaque burden and TCFAs.[25] We observed that most separate lesions either did not change vulnerability or were transformed to a less vulnerable lesion at follow-up (Figures 3 to 5). Although our findings must be interpreted with caution, they support that aerobic exercise combined with optimal medical treatment has a beneficial effect on plaque vulnerability. Whether aerobic exercise, through plaque modification, translates into reduced risk of coronary events needs to be explored.

One study patient had a cerebral hemorrhage 36 hours after completion of an AIT session. Although this event is not enough to estimate risk, and the time span renders increased blood pressure from exercise, an unlikely cause, vigorous exercise has been identified as a trigger for rupture of intracranial aneurysms.[26] However, physical activity in general is associated with decreased risk of cerebrovascular disease.[27]

Our study has a limited sample size. However, the study design with interventional procedures as part of the protocol means obvious limitations and the number of patients included is higher than in several other exercise studies. Also, there was some imbalance in statin use between groups at baseline. It could be argued that this has confounded our results, both with respect to potential group differences and the mechanism for the antiatherogenic effects observed. However, there were no differences in the reduction of necrotic core or plaque burden between different statin users with or without inclusion of exercise group affiliation. Furthermore, low-density lipoprotein cholesterol and C-reactive protein levels were essentially unchanged at follow-up, indicating a modest statin effect during the study period. In the Reversal of Atherosclerosis with Aggressive Lipid Lowering trial, no atheroma regression was observed after 2 different statin regimens administered for 18 months.[28] We, therefore, argue that the reduction of plaque burden and necrotic core observed in our trial could be exercise induced. Strengths of our study include the use of an independent and blinded core laboratory and well validated, supervised exercise protocols[13–15] that do not compromise recommendations in guidelines or patient safety.[29]

Disclosures

The authors have no conflict of interest to disclose.

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Paper II

Paper III