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Results from a randomized clinical trial

Doctoral thesis

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SAMMENDRAG

Effekt av høyintensiv intervalltrening på aterosklerotiske plakk i koronarkar – resultater fra en randomisert studie.

Åreforkalkning (aterosklerose) er en kronisk, progressiv sykdom der kolesterol og ulike betennelsesceller avleires lagvis i blodåreveggen og fører etter hvert til dannelsen av fettputer (plakk) og forkalkninger. Når hjertets pulsårer (koronararterier) rammes, kan det etter hvert føre til forsnevringer som reduserer hjertemuskelens blodtilførsel. Ved etablert koronarsykdom er plakkstørrelse og plakksammensetning viktige prediktorer for videre sykdomsutvikling. Spesielt er store plakk med høyt fettinnhold sårbare da disse har økt risiko for å sprekke og danne blodpropper som helt eller delvis kan blokkere blodsirkulasjonen og forårsake hjerteinfarkt. For å forebygge plakkutvikling er sunn livsstil viktig. Grad av fysisk form, målt som maksimalt oksygenopptak, er en sterk og uavhengig prognostisk faktor for utvikling og prognose av koronarsykdom. Regelmessig trening er derfor et viktig forebyggende tiltak, og treningsbasert hjerterehabilitering har den sterkeste anbefaling i gjeldende retningslinjer. Det er beskrevet mange gunstige effekter av trening på hjertet, men det er begrenset kunnskap om hvordan fysisk trening påvirker koronare plakk.

Dette arbeidet er basert på en klinisk, randomisert, kontrollert treningsstudie; *Impact of cardiac exercise training on lipid content in coronary atheromatous plaques evaluated by near-infrared spectroscopy: A randomized trial (CENIT)*. Studiens hensikt var å undersøke effekten av høyintensitets intervalltrening (HIIT) på koronare plakk hos pasienter med stabil koronarsykdom som fikk utført stentbehandling av trange koronararterier. Nær-infrarød spektroskopi (NIRS) og intravaskulær ultralyd (IVUS) ble brukt som intrakoronar avbildningsmetode. Etter fullført ergospirometri med måling av maksimal hjertefrekvens og oksygenopptak, ble pasientene fordelt i to grupper: en gruppe som gjennomførte to ukentlige økter med supervisert HIIT, og en gruppe som fulgte gjeldende retningslinjer for forebyggende behandling uten spesiell oppfølging. I tillegg til råd om supplerende styrketrening, ble treningsgruppen utstyrt med en aktivitetsmåler for å motivere og oppmuntre til økt treningsmengde. Etter seks måneders oppfølgingstid ble pasientene undersøkt på ny med intrakoronar avbildning, blodprøver, ergospirometri og klinisk undersøkelse.

Avhandlingen består av 3 artikler:

Artikkel I: I denne studien undersøkte vi hvordan regelmessig HIIT påvirket plakksammensetning i koronarkar. Ved bruk av NIRS ble plakkets lipidinnhold målt ved studiestart og etter 6 måneder. Vi observerte ingen signifikante forskjeller i lipidinnhold mellom gruppene. Det var imidlertid en signifikant korrelasjon mellom økning i maksimalt oksygenopptak (uavhengig av gruppetilhørighet) og reduksjon av lipidinnhold.

Artikkel II: I denne studien undersøkte vi hvordan regelmessig HIIT påvirket plakkgeometri i koronarkar. Ved bruk av IVUS ble plakkvolum målt ved studiestart og etter 6 måneder. Vi observerte en signifikant gruppeforskjell. Plakkvolumet ble signifikant redusert i HIIT-gruppen, mens der var en ikke-signifikant økning i plakkvolum i kontrollgruppen.

Artikkel III: I denne studien analyserte vi data fra CENIT-populasjonen ved inklusjon. Hensikten var å undersøke mulig sammenheng mellom sirkulerende lipoprotein-subfraksjoner, målt ved hjelp av kjernemagnetisk resonans (NMR) spektroskopi, og lipidinnhold i koronare ateromatøse plakk målt ved NIRS. Lipoprotein(a) (Lp(a)) og fritt kolesterol i de minste HDL-subfraksjonene (HDL-4) hadde det største potensialet for prediksjon av lipidinnhold i koronare plakk. Etter justering for kardiovaskulære risikofaktorer i modellen var ingen av lipoprotein-subfraksjonene signifikant assosiert med lipidinnhold i koronare plakk.

Oppsummert viser studiene at regelmessig HIIT har en gunstig effekt på koronare plakk. Dette inkluderer både plakkregresjon og til en viss grad plakkstabilisering, i alle fall hos pasienter som forbedrer sitt maksimale oksygenopptak. Lipidinnhold i koronare plakk kunne ikke predikeres ved hjelp av sirkulerende lipoprotein-subfraksjoner i vår studiepopulasjon. Våre resultater bidrar til økt forståelse av de underliggende mekanismene for gunstig effekt av trening på koronare plakk og styrker kunnskapsgrunnlaget for å anbefale trening også for hjertepasienter med stabil koronarsykdom.

"*The heart cannot see what shoes you are wearing, but it can feel the effects."*

Andre Le Gerche

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Institutt for sirkulasjon og billeddiagnostikk, Fakultet for Medisin og Helsevitenskap, NTNU

Veiledere: Professor Rune Wiseth, professor Ulrik Wisløff og førsteamanuensis Erik Madssen

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Fond for hjerteforskning, St. Olavs hospital

SUMMARY

Effect of high-intensity interval training on atheromatous plaques in coronary arteries – results from a randomized trial

Atherosclerosis is a chronic, progressive disease characterized by the deposition of cholesterol and various inflammatory cells within the vessel walls, leading to fatty deposits (plaques) and calcifications. When the coronary arteries, which supply blood to the heart muscle, are affected, it can result in lumen narrowing with reduced coronary artery flow. Plaque size and composition are important predictors of coronary heart disease progression, symptoms, and outcomes. Large plaques with high lipid content are particularly vulnerable, as they carry an increased risk of rupture and the formation of blood clots with the risk of myocardial infarction. Healthy lifestyle is of importance to prevent plaque progression. Physical fitness, expressed as maximal oxygen uptake, has been demonstrated to be an independent prognostic factor for the development and prognosis of coronary artery disease. Accordingly, regular exercise is an essential part of the preventive strategy, and participation in exercise-based cardiac rehabilitation is strongly recommended in current guidelines. Although numerous beneficial effects of exercise on the heart are described, knowledge of how physical exercise affects coronary plaques remains limited.

This thesis is based on a clinical, randomized, controlled exercise study, *"Impact of cardiac exercise training on lipid content in coronary atheromatous plaques evaluated by near-infrared spectroscopy: A randomized trial (CENIT),"* which aimed to investigate the effects of high-intensity interval training (HIIT) on coronary plaques in patients with stable coronary artery disease undergoing Percutaneous Coronary Intervention (PCI). Intracoronary imaging was performed using near-infrared spectroscopy (NIRS) and intravascular ultrasound (IVUS). After PCI, a baseline cardiopulmonary exercise test was performed to measure maximum heart rate and oxygen uptake. Patients were randomly assigned to either the intervention group, which participated in twice-weekly supervised HIIT sessions, or the control group, which followed the current preventive treatment guidelines without additional monitoring. In addition to receiving guidance on supplementary strength training, the intervention group was provided with an activity monitor to motivate and encourage increased exercise volume. After a 6-month follow-up period, intravascular imaging, blood sampling, exercise tests, and clinical data were repeated.

The thesis consists of three articles:

Article I: In this study, we examined the impact of HIIT on plaque composition in coronary arteries. Using intracoronary imaging with NIRS, plaque lipid content was measured at study initiation and after six months. When comparing changes in lipid content, no significant group differences were detected.

There was a significant correlation between increase in oxygen uptake (independent of group allocation) and reduction in plaque lipid content.

Article II: In this study, we investigated how regular HIIT affects plaque geometry within coronary arteries. Using IVUS, plaque volume was measured at the study initiation and again after a six-months period. A significant distinction emerged after comparing the changes in plaque volume between the groups. A favorable plaque regression was demonstrated in the group following HIIT, as opposed to a non-significant increase in plaque volume in the control group.

Article III: In this study, we analyzed baseline data from the CENIT population to explore a potential association between circulating lipoprotein subfractions assessed by nuclear magnetic resonance spectroscopy and coronary plaque composition assessed by near-infrared spectroscopy. Lp(a) and free cholesterol in the smallest HDL subfractions (HDL-4) exhibited the strongest potential for predicting lipid content in coronary plaques. However, after adjusting for cardiovascular risk factors in the model, none of the lipoprotein subfractions were significantly associated with lipid content in coronary plaques.

In summary, these studies demonstrate that supervised HIIT has beneficial effects on coronary artery disease, comprising both plaque regression and, to some extent, plaque stabilization, at least in patients improving their cardiopulmonary fitness. The prevalence of lipid-rich coronary plaques in our study population could not be denoted using circulating lipoprotein subfractions. The results of these studies contribute to a greater understanding of the underlying mechanisms concerning the effect of exercise on coronary plaques and strengthen the scientific evidence for recommending exercise as medicine for patients with stable coronary artery disease.

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Elisabeth Kleivhaug Vesterbekkmo, December 2023

CONTENTS

LIST OF PAPERS

The original articles in this thesis are listed below and referred to by their roman numerals.

Paper I

Vesterbekkmo EK, Madssen E, Aamot Aksetøy IL, Follestad T, Nilsen HO, Hegbom K, Wisløff U, Wiseth R. *J Am Heart Assoc*. 2022 May 17;11(10):e024705. doi:10.1161/JAHA.121.024705. Epub 2022 May 16. PMID: 35574968; PMCID: PMC9238565. **CENIT (Impact of Cardiac Exercise Training on Lipid Content in Coronary Atheromatous Plaques Evaluated by Near-Infrared Spectroscopy): A Randomized Trial.**

Paper II

Vesterbekkmo EK, Aamot Aksetøy IL, Follestad T, Nilsen HO, Hegbom K, Wisløff U, Wiseth R, Madssen E. *Eur J Prev Cardiol.* 2023 Mar 27;30(5):384-392. doi:10.1093/eurjpc/zwac309. PMID: 36562212. **High-intensity interval training induces beneficial effects on coronary atheromatous plaques - A Randomized Trial.**

Paper III

Sæther JC, Vesterbekkmo EK, Gigante B, Giskeødegård GF, Bathen TF, Follestad T, Wiseth R, Madssen E, Bye A. *Int J Cardiol Heart Vasc*. 2023 May4;46:101215.doi:10.1016/j.ijcha.2023.101215. PMID: 37255857; PMCID: PMC10225625. **The association between circulating lipoprotein subfractions and lipid content in coronary atheromatous plaques assessed by near-infrared spectroscopy.**

ABBREVIATIONS

BMI	Body mass index
CAD	Coronary artery disease
CI	Confidence interval
CPET	Cardiopulmonary exercise testing
CVD	Cardiovascular disease
HIIT	High-intensity interval training
IVUS	Intravascular ultrasound
LCBI	Lipid core burden index
$maxLCBI_{4mm}$	The maximum value for lipid core burden index for any 4mm region
MET	Metabolic equivalent task
NIRS	Near-Infrared Spectroscopy
NMR	Nuclear magnetic resonance
PCI	Percutaneous coronary intervention
ROI	Region of interest
VO_{2peak}	Peak oxygen uptake
WSS	Wall shear stress
HR_{peak}	Peak Heart Rate

1. INTRODUCTION

1.1 General background

Cardiovascular disease (CVD) stands as a major global health challenge, responsible for about a third of all worldwide deaths, making it the leading cause of mortality[1]. Furthermore, when considering both premature deaths and disability-adjusted life-years, CVD ranks high in terms of its impact on overall global disease burden[2]. However, in high-income countries, the mortality of coronary artery disease (CAD), a subset of CVD, has shown a rapid decline since the 1980s. This reduction can be attributed to effective intervention strategies such as enhanced tobacco control, blood pressure-lowering and cholesterol-lowering treatments. Additionally, advances in medical procedures and acute- phase treatment of CAD have markedly improved patient outcomes[3]. Despite the expanding use of medical and surgical therapies, CAD remains an enduring public health challenge. This underscores the ongoing need for maintaining efforts in areas like early risk detection, risk factor management and improved accessibility to healthcare providers and services[4].

1.1.1 Coronary atherosclerosis

Atherosclerotic CAD is the most common form of CVD. It is characterized by the pathological process of lipid accumulation and inflammation within the arterial walls of the epicardial arteries potentially resulting in a clinical event. It is a chronic inflammatory process that progresses slowly and exhibits significant heterogeneity[5]. CAD encompasses a wide range of disease manifestations, starting from the accumulation of minor lipid deposits in the inner layer of the artery (sub-intimal) and progressing to the development of large plaques that restrict blood flow. Age, sex, and genetic predisposition are non-modifiable risk factors that play a crucial role in determining the risk of developing CAD[4]. Furthermore, there are several primary causal and modifiable risk factors that contribute significantly to the onset of the disease. These include elevated levels of apolipoprotein-B-containing lipoproteins (Apo-B), with low-density lipoprotein (LDL) being the most prevalent lipid, high blood pressure (BP), cigarette smoking, and diabetes mellitus (DM). Other important risk factors are physical inactivity and adiposity, or excess body fat, which increases the risk of CVD through both major conventional risk factors and other mechanisms. While the aforementioned risk factors affect both men and women, there are also gender-specific ones to consider. Migraine, polycystic ovary syndrome (PCOS), hypertensive disorders of pregnancy (such as preeclampsia), gestational diabetes, preterm delivery, and premature menopause are all risk enhancers in women[6,7]. On the other hand, erectile dysfunction, male pattern baldness, and testosterone deficiency are conditions known to increase the risk in men[6]. Moreover, there are additional modifiers and clinical conditions that influence the development of CAD[4]. The precise extent to which these factors contribute to the incidence and mortality is still subject to ongoing debate.

1.1.2 Management of patients with stable angina pectoris

Patients with established CAD are considered to be at a very high risk of new events[5]. CAD exhibits long periods of stability but can become unstable unexpectedly, often due to an acute atherothrombotic event triggered by plaque rupture or erosion. Nevertheless, CAD is fundamentally a chronic condition that tends to progress over time, and patients remain at increased risk for further disease progression, even when no obvious symptoms are present. A healthy lifestyle, medical therapy, and myocardial revascularization constitute the three essential pillars for treating ischemic heart disease. The long-term strategies for avoiding recurrent events after PCI of stable CAD, are highlighted in Figure 1. Addressing lifestyle modifications, such as adopting a healthy diet, engaging in regular exercise training, smoking cessation, psychosocial management/stress reduction, and maintaining a healthy weight, can positively impact the progression of CAD[5,7]. Optimal pharmacological treatment, including anti-thrombotic therapy, lipid-lowering therapy, and promoting good drug adherence and persistence, is mandatory. Annual influenza vaccination is also recommended. To obtain risk factor treatment goals is important, including a systolic blood pressure < 130 mmHg if tolerated , LDL-C < 1.4 mmol/L, and HbA1c < 53 mmol/mol in diabetic patients[7]. In accordance with the current guidelines, following these recommendations decreases the risk of disease progression, future revascularizations, and acute coronary events[4,5,7].

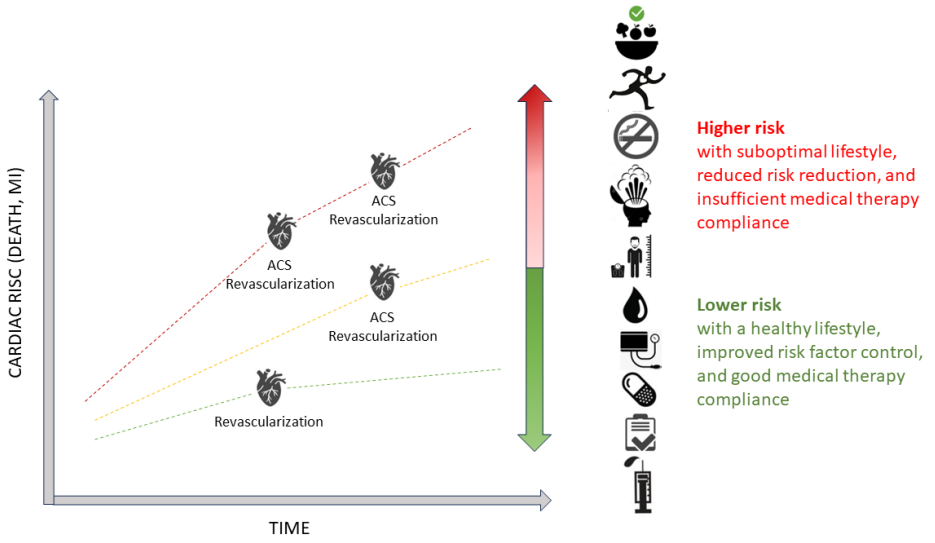

Figure 1. An overview illustrating a typical history of chronic coronary syndromes. MI; Myocardial infarction. Modified version from the original figure from Knuuti et al.[5] and Byrne et al.[7].

Revascularization procedures, such as angioplasty or bypass surgery, may be recommended to restore blood flow to the affected coronary arteries. Myocardial revascularization is essential in angina symptom relief and may improve prognosis for a subset of patients. Prior trials comparing optimal medical therapy with or without revascularization, have not demonstrated a reduction in cardiovascular events in patients with stable ischemic heart disease. The recent ISCHEMIA (International Study of Comparative Health Effectiveness with Medical and Invasive Approaches) trial[8] aimed to assess the effect of an initial invasive strategy, which included revascularization (such as angioplasty or bypass surgery) in addition to optimal medical therapy, compared to an initial conservative strategy involving only optimal medical therapy for patients with stable ischemic heart disease. Also, this study failed to demonstrate a significantly better outcome following an initial invasive strategy. The primary endpoint, a composite of death from cardiovascular causes, nonfatal myocardial infarction, resuscitated cardiac arrest, or hospitalization for unstable angina or heart failure, did not significantly differ between the two groups.

1.1.3 Plaque morphology

The association between the burden of coronary atherosclerosis and clinical outcomes is well-established, both from necropsy- and registry studies[9]. Complementing this, large datasets of patients undergoing coronary angiography have shown a correlation between more severe or extensive narrowing of the arterial lumen and adverse cardiovascular outcomes[10,11]. Thus, the presence of atherosclerotic plaques serves as the underlying pathological substrate for the occurrence of ischemic cardiovascular events. There is an increasing interest in plaque characterization using various imaging techniques to further understand the phenotype, extent, and progression of plaques, and their association with the prospective risk of cardiovascular events[12-14].

Atherosclerotic plaques can be classified into two phenotypes: stable or unstable plaques. Stable (calcified) plaques are characterized by a small lipid pool, low concentrations of inflammatory cells, and a thick stabilizing fibrous cap. In contrast, unstable and mixed plaques exhibit a large lipid pool, high inflammatory activity, and a thin fibrous cap, making them more vulnerable to rupture and triggering acute cardiovascular events[15-19]. CAD may be stable for long periods but can become unstable at any time, typically due to increased inflammatory activity or shear stress, resulting in plaque rupture or erosion[5]. Muller et al. first used the term vulnerable plaque in the 1980s[20]. These coronary plaques possess specific morphological features making them more susceptible to rupture[20,21]. Autopsy studies have shown that most ruptured plaques exhibit a common phenotype known as thin cap fibro atheroma (TCFA). TCFAs are characterized by an increased plaque burden, positive remodeling, a large lipid-core covered by a thin fibrous cap, increased macrophage accumulation, and the presence of neovascularization[22]. A study combining near-infrared spectroscopy (NIRS) and intravascular ultrasound (IVUS) showed that non-obstructive mild lesions with high lipid content and high plaque burden are most likely to cause future adverse cardiac events following percutaneous

coronary intervention (PCI) for the culprit and hemodynamic significant lesions[23,24]. Moreover, the Lipid Rich Plaque study[25] indicated that lipid-rich plaques of non-culprit lesions are associated with subsequent major adverse coronary events in patients with CAD.

1.2 Invasive imaging of coronary artery disease

1.2.1 Coronary angiography

Coronary angiography, as an invasive diagnostic procedure, allows visualization of coronary arteries and is regarded as the gold standard for diagnosing CAD and guidance of coronary artery revascularization[26,27]. Coronary angiography is preferable when the suspicion of established CAD is high, given the possibility of performing an intervention during the same procedure[27]. Although low, the risk of complications with invasive angiography is not negligible. By mainly visual assessment, the severity of the coronary narrowing is reported with 50% diameter stenosis defined as a significant stenosis. However, coronary angiography has major limitations in assessing the hemodynamic significance of a coronary artery stenosis, as lesion length, the magnitude of the territory being served, and other factors that are of importance for the hemodynamic significance[28,29]. With moderate lesions or diffuse disease, the diagnostic accuracy is limited for the above-mentioned reasons. In daily practice, intracoronary pressure recordings are used to overcome these limitations in defining hemodynamic significance[28]. For a more detailed evaluation of CAD, including plaque morphology, and to guide interventions, novel intravascular imaging techniques are established.

1.2.2 Intravascular imaging

During the last three decades, the technological development of intravascular imaging techniques has been significant and enabled a comprehensive assessment of atherosclerosis beyond the "lumenogram" provided by coronary angiography. The development of different imaging modalities has enabled a thorough evaluation of the vessel wall[13,30]. Thus, essential information regarding atherosclerotic disease burden, progression, plaque composition, and vulnerability has been provided. Intravascular imaging is performed using special catheters, and currently, IVUS, NIRS, optical coherence tomography (OCT), or a combination catheter of these modalities are available. Coronary intravascular imaging has provided novel insights into the development, progression, and treatment of coronary artery disease. The different modalities can be performed before, during, and after PCI to determine the need for coronary revascularization and to assist, plan, optimize, and evaluate the result of the intervention[30].

Intravascular ultrasound (*IVUS*)

IVUS was first developed in the early 1970s, and during the last decades, its use has increased. This sound-based imaging modality utilizes an intravascular catheter with a piezoelectric crystalline

transducer generating ultrasound pulses to provide real-time 360° cross-sectional images[31]. The automated pullback of the transducer at a constant rate enables lesion length measurements. Current generation IVUS models typically range from 20 to 60 MHz, depending on the catheter system used. The spatial resolution depends on the ultrasound pulses' wavelength and beam width. Tissue penetration is determined by frequency, and as frequency is increased, penetration distance is decreased[32]. Within the vessel wall, the different structures vary in echogenicity. Calcifications and fibrous tissue produce brighter hyperechoic signals, while echo-lucent structures, such as lipid collections, produce low-intensity hypoechoic signals. Based on differences in the reflection of ultrasound waves, the grayscale cross-sectional image of the target vessel is generated[31,33]. IVUS enables high-resolution imaging of the coronary artery wall. With a thorough assessment of the luminal outline and external elastic lamina, the accurate quantification of plaque burden and characterization of intracoronary pathology, plaque morphology, and vessel wall architecture is permitted[32,34]. Lesions in patients with stable angina demonstrate less expansive remodeling by IVUS than in ACS patients, suggesting that expansive remodeling might be associated with plaque vulnerability. Additionally, complex, unstable plaques are typically bulky, eccentric, and positively remodeled and exhibit disruption features, including ulceration, intimal flap, and thrombus[34]. Additional advantages of IVUS, as a supplement to angiography, include pre-intervention planning, peri-procedural guidance, and post-procedural evaluation. With plaque imaging, composition and essential lesion characteristics can be assessed, reference segments and landing zones can be identified, and stent size selection optimized[35,36].

Near-infrared spectroscopy (NIRS)

The effort to identify vulnerable plaques prone to rupture has been ongoing for more than 30 years. In this regard, the development of the near-infrared spectroscopy method represents a substantial methodological advancement as it demonstrates high reliability[16,37] and reproducibility[38,39] in detecting and quantifying the amount of lipid content in coronary plaques. NIRS has been extensively validated and approved by the U.S. Food & Drug Administration in 2008. The ability to identify lipid-rich plaques based on spectral signatures, differentiates NIRS from other intravascular imaging modalities[34,40].

NIRS is a unique imaging modality that relies on the property of substances to absorb and scatter near-infrared light with wavelengths from 800 to 2500 nm. The NIRS system comprises a catheter, a scanning near-infrared laser, and an automatic retraction and rotation device. Using electromagnetic radiation, a light-source emits near-infrared light towards the target. The proportion of diffusely reflected light returning to a detector is analyzed as a function of wavelength[34,40,41]. Based on the different light absorption in the near-infrared spectrum and the specific spectral signals produced in this process, chemical composition of coronary plaques can be determined. The intravascular imaging is performed with an imaging catheter delivered to the distal portion of the target vessel over a standard coronary guidewire. At the tip of the catheter, the dual imaging source comprising NIRS and IVUS is located.

During the imaging process, the light source is rapidly rotated while the catheter is automatically withdrawn through the vessel by a motorized pullback system. Accordingly, images of the total circumference of the vessel are built during pullback. Returning scattered spectral signals are detected by the catheter and transmitted further to the computer housed in a bedside console where software analyzes the spectral data. A color-coded graphical representation, a chemogram, is constructed with the x-axis representing axial position along the length of the artery, and the y-axis representing circumferential position.

The chemogram varies from red to yellow, where yellow pixels represent the highest probability of lipid deposition. To provide a quantitative estimation of the amount of lipid present in non-stented segments of the target artery, the lipid core burden index (LCBI) can be calculated, and the formula is often expressed as:

$$\text{LCBI} = (\text{Number of Lipid Pixels} / \text{Total Number of Pixels in Region}) \times \text{Scaling Factor}$$

The scaling factor, often multiplied by 1000, ensures that LCBI values fall within a standardized range. Accordingly, LCBI represents the amount of lipids in the arterial segment scanned[34,40,41].

Max4mmLCBI (maxLCBI$_{4mm}$) refers to the maximum LCBI within any 4mm segment of a scanned arterial region. MaxLCBI$_{4mm}$ is a well-used parameter in intravascular imaging research. Numerous studies have consistently demonstrated its capacity to contribute valuable insights into a patient's cardiovascular risk, particularly regarding the detection of vulnerable plaques. Moreover, maxLCBI$_{4mm}$ is an important tool for evaluating the efficacy of different interventions in influencing the composition of atheromatous plaques[23,42-47].

Among the first clinical studies suggesting that NIRS can identify vulnerable plaques are results from cross-sectional studies showing that compared to patients with stable angina, lesions responsible for acute coronary syndromes (ACS) more commonly exhibited lipid-rich plaques. Furthermore, lipid-rich plaques were more prevalent in ACS patients compared to patients with stable angina[44]. Later, several trials have demonstrated that lipid content, measured as maxLCBI$_{4mm}$, in coronary plaques is a valid risk factor for subsequent acute coronary events[19,23,42,43].

Although NIRS may identify vulnerable plaques, the exact role of NIRS in clinical practice to guide intervention is so far not established in guidelines.

Optical coherence tomography (OCT)

The concept of optical coherence tomography was first reported in 1991 and further intracoronary tested in the early 2000s. OCT catheters are developed to receive and emit near-infrared light waves through a rotating single optical fiber connected with an imaging lens. The signals are converted into real-time high spatial and contrast resolution, cross-sectional, and 3-dimensional volumetric images[30,48]. OCT has the highest resolution among the currently available imaging techniques[34]. This modality provides detailed image quality with the ability to distinguish the lumen-intima interface. Moreover, an enhanced definition of the intima, media, and fibrous cap is permitted, and a thorough assessment of plaque morphology at the expense of a lower penetration depth[48]. The excellent resolution also allows for the characterization of in-stent re-stenotic lesions, including the ability to detect neo-atherosclerosis. OCT enables the precise location of side branches, wire location, and stent visualization and holds greater sensitivity to detect intimal/medial dissections compared with IVUS[34,49]. This imaging method is not utilized in this thesis.

Table 1 summarizes the properties of the different invasive imaging of CAD described above.

Table 1. Comparison of IVUS, NIRS and OCT imaging modalities

	IVUS	NIRS	OCT
Energy source	Ultrasound	Near-infrared light	Near-infrared light
Wavelength, μm	35-80	0.75 -1.4	1.3
Resolution, μm	40-200 (axial) 200-300 (lateral)	NA	15-20 (axial) 20-40 (lateral)
Tissue penetration, mm	10	1	1-2.5
Blood clearing	Not required	Not required	Required
Advantages	More favorable imaging in larger vascular structures e.g., left main coronary artery, and ostial lesions May visualize expansive remodeling and calculate plaque geometry Extensive clinical experience → IVUS has been used clinically for almost three decades Extensive research regarding impact of IVUS guidance of the procedural result as well as clinical outcomes	The ability to identify lipid rich and vulnerable plaques	Clear images being easy to interpret with reliable automatic analyses Detailed information of the intima and subintimal structures including plaque morphology including thin-cap fibroatheroma More favorable imaging in severe calcification and detection of thrombus and dissections Enhanced PCI-guidance regarding length, diameter, apposition, and expansion
Disadvantages	Images can be difficult to interpret. Low resolution in the longitudinal view Limited tissue characterization and challenging thrombus detection	The precise depth of the interrogated tissue is not known Lack of structural information	Limited penetration Cannot accurately assess residual plaque burden at stent-edges Need of extra contrast injection

IVUS = intravascular ultrasound; NIRS = near-infrared spectroscopy; OCT = optical coherence tomography. Table is adapted based on references 32, 36, 48, 49.

1.3 Physical activity and exercise training

Physical activity (PA) is defined as any bodily movement resulting from skeletal muscle contraction that increases energy expenditure above the basal metabolism[50]. In clinical studies, the duration of PA is typically reported as minutes/week and the intensity as metabolic equivalents (METs). One MET is defined as the oxygen consumption while sitting at rest and is equivalent to 3.5 mL O_2 kg^{-1} min^{-1} [51]. Light activity, such as slow walking, typically ranges from 1.6 to 2.9 METS. Moderate-intensity activity, like moderate speed walking, is within the range of 3.0 to 5.9 METS. Vigorous activity, such as moderate jogging, is classified as ≥6 METS[51].

To provide a frame of reference, patients who undergo cardiac stress testing and are able to achieve ≥10 METS (for example, moderate to fast jogging) on a treadmill without experiencing ST-depression, are generally considered to be at very low risk for clinical CVD. This indicates a high level of cardiorespiratory fitness (CRF) and suggests a lower likelihood of developing CVD-related complications[52].

As a subcategory of PA, exercise training is defined as any repetitive, structured, and planned intervention to improve or maintain CRF, health, athletic performance, or the combinations thereof[50]. CRF is defined as the capacity of the cardiovascular and respiratory systems to supply oxygen to the working skeletal muscles during PA. CRF can be measured directly, expressed as maximal oxygen uptake (VO_{2max}) or peak oxygen uptake (VO_{2peak}) during a cardiopulmonary exercise test via direct spirometry gas-exchange measurements, and is commonly expressed as mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ or in METs[53]. Although CRF has a genetic component and declines with age[54], it is a surrogate marker of habitual physical activity. The enhancement of cardiac output (CO) is the primary factor influencing CRF and VO_{2max}, as explained by the Fick equation: $VO_2=CO\times a\text{-}vO_{2diff}$, where $a\text{-}vO_{2diff}$ represents the arteriovenous O_2 difference. Cardiac output (CO) is determined by the multiplication of stroke volume (SV) and heart rate (HR), and both of these factors experience significant increases during aerobic exercise. CO remains relatively consistent at approximately 5 $L\cdot min^{-1}$ at rest. However, this can vary significantly during peak exercise, ranging from around 20 $L\cdot min^{-1}$ in apparently healthy, untrained individuals to approximately 40 $L\cdot min^{-1}$ in individuals undergoing regular endurance training. This substantial variation in CO helps account for the broad spectrum of VO_{2peak}, with typical values ranging from about 35 to > 90 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ in athletes[55,56].

1.3.1 Physical activity and the association with CVD

The beneficial association between PA and CAD has been known for decades. Morris demonstrated in 1953 that stair-climbing bus conductors and mail delivery postmen had half the rate of CAD events compared with their sedentary bus driver and clerical postal worker counterparts[57]. Since then,

substantial epidemiological, clinical, and basic science evidence suggest that regular physical activity, higher cardiorespiratory fitness, or both, delay the development of atherosclerotic CVD and reduce the incidence of CHD events[53,58-60]. In a meta-analysis from 1987, Powell described the association between PA and CHD incidence from 43 studies[61]. The relative risk of CHD corresponding to physical inactivity ranged from 1.5 to 2.4, with a median value of 1.9. Additionally, the relative risk of a passive lifestyle appeared to be similar to that associated with other established CHD risk factors. Another systematic review and meta-analysis of 33 PA studies (n=883 372 participants) reported a pooled risk reduction of 35% and 33% for CVD and all-cause mortality, respectively, among the most physically active cohorts[62]. As highlighted in the 2020 World Health Organization PA Guidelines[63], emerging new evidence indicates that high levels of sedentary behavior are associated with CVD and type 2 diabetes, and cardiovascular and all-cause mortality. For this reason, the World Health Organization and other health authorities [4,27] advise adults to engage in at least 150 to 300 minutes/week of moderate-intensity PA or 75 to 150 minutes/week of vigorous-intensity aerobic PA, or combinations thereof, throughout the week.

1.3.2 CRF as a risk factor and modulator of CVD- and all-cause mortality

Over the past two decades, a substantial body of evidence has emerged indicating that low CRF levels are associated with a significantly increased risk of CVD and all-cause mortality[64-66]. As highlighted by Ross et al.[67] these observations are based on different populations comprising healthy men and women, individuals with suspected or known CVD, and those with comorbid conditions, such as lipid abnormalities, hypertension, type 2 diabetes, and obesity. Given that CRF has proven to be a robust predictor of mortality, probably even more robust than established risk factors for CVD, incorporating CRF into risk assessment scores substantially improves the ability to reclassify individuals' risk for adverse outcomes. Surprisingly, even though physical inactivity and low levels of CRF have been established as markers of cardiovascular health, it remains the only major risk factor not routinely assessed in clinical practice[66,68].

Previous studies involving individuals with established atherosclerotic risk factors have shown a 12–20% decrease in cardiovascular events per 1 MET achieved, with a continuous inverse correlation up to 12 METS. Current literature has demonstrated a comparable impact in healthy individuals, with an incremental benefit continuing even beyond 12 METs, suggesting no upper threshold for cardioprotective effects of exercise[69]. The strength of the association between CRF and mortality was also supported in a meta-analysis conducted by Kodama and colleagues[66], pooling data from 33 studies with 103 000 participants. In line with this meta-analysis, every 1 MET increase in aerobic capacity was found to be associated with a 13% decrease in the risk of all-cause mortality and a 15% reduction in coronary artery disease events This meta-analysis also exemplified the prior finding that the most

significant mortality benefits emerge when advancing from the least fit and the next least fit group; fewer advances in health outcomes were registered when individuals in the moderate- to high-fit groups were compared.

1.3.3 Cardiac rehabilitation

Cardiac rehabilitation (CR), with exercise training as the central element, is considered essential to the contemporary care of patients with CAD and has been given a Class Ia recommendation from the European Society of Cardiology, the American Heart Association, and the American College of Cardiology[4,5,7,70]. CR comprises a comprehensive set of multidisciplinary core components where secondary prevention is most effectively provided. These elements include patient assessment, managing and controlling cardiovascular risk factors, guidance on physical activity, dietary advice, tobacco cessation counseling, patient education, psychosocial support, and vocational assistance[71]. An updated Cochrane Review from 2021, which incorporated data from 85 trials (n= 23,430 patients with CAD), reaffirmed the earlier findings that exercise-based CR provides essential benefits, including reduced risk of myocardial infarction, a likely slight reduction in all-cause mortality, a substantial decrease in all-cause hospitalization rates, along with associated healthcare costs, and an enhancement in health-related quality of life for up to 12 months of follow-up. Over longer-term follow-up, benefits may include reductions in myocardial infarction and cardiovascular mortality[72].

Exercise programs in CR are designed to enhance participants' CRF and muscular strength. However, it is worth noting that international CR guidelines vary in their recommendations regarding exercise intensity [73 71]. Evidence gathered over the years have revealed that the magnitude of changes in exercise capacity following CR is highly variable. According to the most recent position paper from the Secondary Prevention and Cardiac Rehabilitation Section of the European Association of Preventive Cardiology, the general recommendation is to aim for a moderate or moderate-to-high exercise intensity level whenever feasible. Alternatively, individualized recommendations should be considered based on the patient's characteristics and the nature of their medical condition[71]. Recent research findings indicate that HIIT, characterized by exercise at or above 85% of VO_{2peak}, 85% of Heart Rate Recovery, or 90% of Peak Heart Rate (HR_{peak}) alternated with periods of lower-intensity exercise, may yield superior results compared to moderate-intensity continuous training (typically defined as exercise at 50–75% of VO_{2peak}, 50–75% of Heart Rate Recovery, or 50–80% of HR_{peak} in enhancing CRF among individuals diagnosed with CAD[73].

During the recent years, HIIT has gained popularity and has been proven an effective and safe approach to aerobic exercise training in individuals with CAD[74-76]. A thematic review from 2017[77] summarized the CV adaptations to HIIT throughout different patient cohorts, including patients with CHD, heart

failure, heart transplant recipients, and effects on CVD risk factors like metabolic syndrome, hypertension, obesity, and type II diabetes. After this publication, additional studies have demonstrated beneficial health effects after HIIT in patients with atrial fibrillation[78], and in heart failure patients with and without cardiorenal syndrome[79].

There are still conflicting reports on the benefits of HIIT versus MICT in cardiac patients, and controversy remains regarding the optimal exercise prescription[80]. Nevertheless, contemporary studies and current guideline recommendations underscore the need to optimize and individualize exercise prescriptions to maximize improvements in exercise capacity[81].

1.4 Cardiac adaptations to exercise training

The cardiovascular advantages of exercise are multifactorial and comprise essential systemic effects on skeletal muscle, peripheral vasculature, metabolism, and beneficial alterations within the myocardium itself[82]. The positive modifying effects on traditional CVD risk factors, such as lowering blood pressure, improving lipid profiles, reducing adipocyte mass and body mass index, enhancing insulin sensitivity and glucose uptake by skeletal muscle, reducing inflammation, psychological stress, and acting anti-thrombotic, are widely recognized. Additionally, as highlighted in Figure 2, endurance exercise affects the heart directly by encompassing structural, functional, electrical, and vascular changes[83].

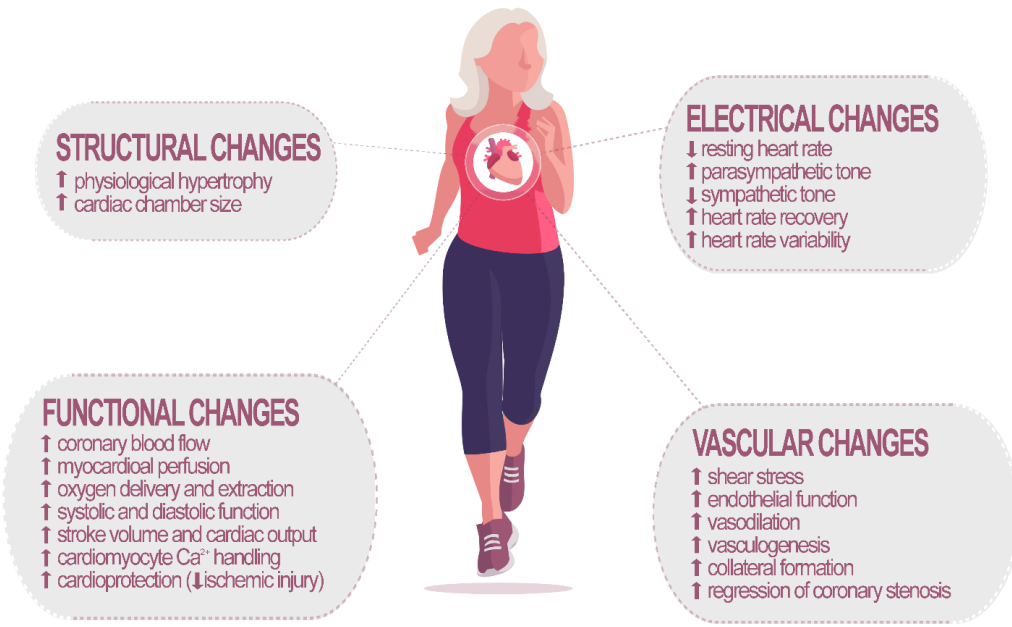

Figure 2. Cardiac effects of endurance exercise training

Exercise-induced cardiac remodeling, commonly called the "athlete's heart," is characterized by increased cardiac chamber size and ventricular wall hypertrophy developed through increased volumes and pressure loads placed on the heart during exercise[84]. Exercise increases coronary blood flow and myocardial perfusion, improving oxygen delivery and extraction. The structural changes are accompanied by enhanced cardiac function, including increased contractility, high cardiac outputs, and early diastolic filling secondary to increased preload and myocardial relaxation. Exercise has been shown to improve cardiomyocyte Ca^{2+} sensitivity and contractility due to a direct upregulation and increased activity of the sarcoplasmic reticulum Ca^{2+}ATPase 2a (SERCA2a) and indirectly via increased phosphorylation of phospholambam (PLB), reducing PLB-mediated inhibition of SERCA2a[82]. Finally, regular exercise has proven to blunt angina symptoms[85]. Exercise training is associated with improved autonomic tone with increased parasympathetic- and decreased sympathetic tone. Additionally, there is an increase in heart rate recovery, enhanced heart rate variability, and reduced resting heart rate. These alterations are predominantly observed in athletes participating in high-intensity dynamic endurance sports[86].

1.4.1 Exercise training and coronary atheromatous plaques

The mechanisms by which exercise training exerts its favorable effects on the vascular system have yet to be fully understood. However, the following factors have been suggested to contribute to the improved myocardial perfusion: improved endothelial function, collateral formation, vasculogenesis, and regression of coronary stenosis[85,87]. With increased coronary blood flow during exercise, a direct effect on the vasculature is exerted due to the impact of repetitive exposure to hemodynamic stimuli, such as shear stress and transmural pressure. These changes may induce alterations in vessel wall function and morphology[83,87-89]. It has been shown in healthy subjects that episodic changes in shear stress provide the principal physiological stimulus to these adaptations in flow-mediated endothelial function and vascular remodeling in response to exercise[88]. Flow-changes modify gene expression with elements in their promoter regions that respond to shear stress[90]. Individuals with higher aerobic capacity and those who have recently completed an aerobic exercise training program, demonstrate notably reduced measures of arterial stiffness. In CVD-patients, the essential functions of the endothelium (selective barrier function, low adhesion to leucocytes, anti-thrombogenicity, and regulation of vascular tone) are impaired[91].

In contrast, regular endurance exercise helps maintain endothelial cell integrity, induce partial restoration, preserve the endothelial barrier function, and counteracts further lipid accumulation in the subendothelial vascular wall, halting the atherosclerotic process and further plaque progression through several mechanisms[87,89]. Some central elements are the improvement in endothelial-mediated vasodilation, improved arterial compliance, and blood pressure reduction due to the upregulation of

nitric oxide (NO) production by vascular endothelial cells[89]. There is a hypothesis that exercise training restores the balance between NO production and inactivation, resulting in an enhanced NO bioavailability associated with a partial restoration of endothelial function[89]. While NO is the most extensively studied endothelium-derived relaxation factor, other factors like prostacyclin and hydrogen peroxide, as well as endothelium-derived constricting factors such as prostanoids and endothelin-1, also play a role in endothelium-dependent vasomotion. However, their influence on endothelial function, the development of CAD, and particularly the impact of exercise training on their regulation remains unexplored and warrants further investigation[85].

Exercise training has been demonstrated to impact angiogenesis directly with collateral formation, enhanced functional coronary/collateral responsiveness, increased arteriolar and capillary density, induced vascular remodeling, vasomotor control adjustments, and act on the coronary arterial wall[89]. Recently, ultrasound- and MR-based imaging have verified that exercise training improves size and dilatation capacity of coronary arteries[89]. This alterations aligns with the concept initially proposed by Morganroth et al.[92] that regular exercise training induces cardiac remodeling also at the vascular level[89,93].

Only a few RCTs have assessed the effect of exercise training on the regression of coronary stenoses. In 1990, The Lifestyle Heart Trial by Ornish et al. was published. This landmark study was one of the first to show the beneficial effects of exercise on plaque regression in patients with CAD using quantitative coronary angiography. In this study, one year of moderate exercise, associated with a low-fat Mediterranean diet, induced a 3.1% regression in coronary stenosis without using lipid-lowering drugs. In contrast, the physically inactive control group demonstrated an 11.8% progression of coronary stenosis[94].

In 1993, Hambrecht et al. demonstrated that regression of coronary lesions after one year was only observed in patients who expended more than 9228 kilojoules per week during exercise. Moreover, it was found that 90% of patients in the training group experienced a halting in the atherosclerotic disease progression, with an average increase of 0.02 mm in the minimal stenosis diameter in the training group, in contrast to a decrease of 0.15 mm in the target lesion within the control group[95].

In 1994, Haskell et al.[96] published The Stanford Coronary Risk Intervention Project, which employed a multifaceted strategy encompassing a low-fat diet, smoking cessation, stress management training, and moderate exercise training, resulted in a significant 49% reduction in cardiovascular events among the 145 patients in the intervention group over a four-year follow-up period. Furthermore, this approach slowed the progression of atherosclerotic narrowing in the coronary arteries, with the target area experiencing a reduction in coronary lumen diameter of 0.024 mm per year, as opposed to the control group, which showed a decline of 0.045 mm per year[96].

In recent years, intracoronary imaging has been increasingly used in diagnostics and research due to its significant advantages. Sixt et al[97]. examined the effects of exercise training in combination with a hypocaloric diet and optimized medical treatment on coronary endothelial function and plaque burden in a small study including patients with CAD and type 2 diabetes mellitus. After a 6-months intervention, there was an improvement in glucose metabolism and coronary endothelial function, while the plaque burden in nonsignificant atherosclerotic lesions remained unchanged. However, the sample size was only 11 vs. 12 patients.

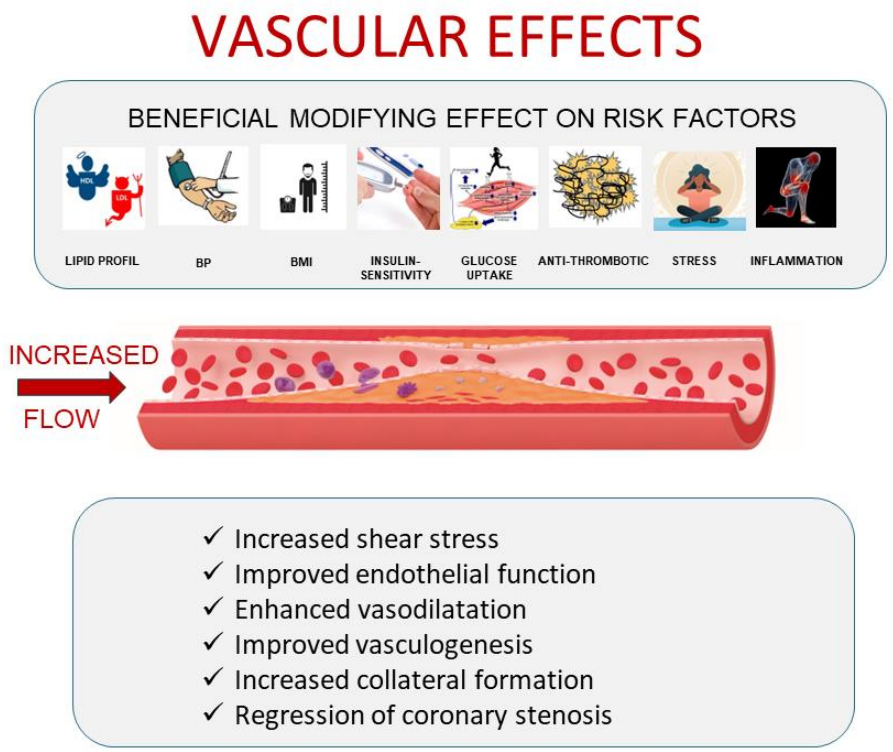

Figure 3. Effects of endurance exercise training on risk factors and the vessel wall. BP: Blood pressure; BMI: Body mass index

In a previous study from our research group, Madssen et al.[98] assessed the effects of HIIT vs. moderate continuous training on coronary atherosclerosis in patients with CAD evaluated by grey-scale- and radiofrequency-IVUS. After 12 weeks of exercise training, the change in plaque structure and morphology did not differ between the two groups. On the other hand, regular aerobic exercise and

optimal medical treatment reduced necrotic core in both groups in defined coronary segments. An insignificant 10.7% reduction in plaque burden across separate lesions, regardless of the intervention group, was observed ($p = 0.06$). These findings were reproduced in a small IVUS study by Nishitani-Yokoyama et al.[99], demonstrating a correlation between exercise volume and a reduction in plaque and lipid volume, but no significant difference in the change of plaque regression or components between the intensive and standard CR groups.

Despite these studies, data regarding the effect of exercise training on plaque volume, plaque composition, and vulnerability are modest, and no previous trial has investigated the effect of exercise training on lipid content in coronary arteries. Therefore, this thesis's main objective was to assess exercise-induced effects on atherosclerotic plaque using NIRS and IVUS.

1.5 Surrogate biomarkers for lipid content in coronary plaques

The identification of non-invasive surrogate biomarkers for vulnerable plaques is of great clinical interest. As coronary plaques are a key pathological feature of atherosclerosis, the composition, particularly the amount of lipid content, plays a crucial role in determining their stability and likelihood of causing adverse cardiovascular events[13]. Traditionally, invasive imaging techniques have been used to assess plaque characteristics. However, as these methods are invasive and carry some risks as well as increases the costs, their widespread use in routine clinical practice is limited. Thus, the development of biomarkers for lipid content in coronary plaques has the potential to improve risk stratification and treatment decisions for patients with CAD. Finding such predictable biomarkers may help identify individuals at higher risk for plaque rupture and cardiovascular events, enabling more targeted preventive treatments, lifestyle modifications, and personalized interventions.

1.5.1 Lipoprotein subfractions

Circulating lipoproteins are highly heterogeneous in particle structure, density, content, biological activity, and intravascular metabolism[100]. Using lipidomics, a detailed characterization allows for a more comprehensive assessment of the lipoprotein profile and its potential implications for cardiovascular risk. The advancements in lipidomics in recent years have enabled research efforts to unravel lipid dysregulation in CAD, enabling an understanding of the underlying pathophysiological mechanisms and identification of predictive biomarkers beyond traditional lipids. However, it is still unknown how and which lipid molecular species affect the risk of CAD and enhance CVD risk prediction in addition to the traditional serum lipid biomarkers[101].

Kishimoto et al. first used the term "lipidomics" in 2001[102]. Two years later, Han and Gross drafted the definition for this emerging discipline[103]. This coincided with the inception of LIPID MAPS. In 2022,

The International Lipid Classification and Nomenclature Committee, with sponsorship of the LIPID MAPS Consortium, established a "Comprehensive Classification System for Lipids" based on well-defined chemical and biochemical principles together with ontology[104].

1.5.2 Nuclear Magnetic Resonance

NMR has emerged as a pivotal tool for investigating the structure of lipoprotein particles since the first publication by Steim et al. in 1968[105]. Subsequently, the PubMed database has registered over a thousand publications under the search term "NMR and lipoprotein," attesting to the widespread application and continued relevance of NMR in this field.

Each class of lipoproteins, LDL, HDL, and VLDL, produces a distinct NMR signal with a unique spectral line shape, enabling clear differentiation. Additionally, precise quantification of particle numbers is achievable, as the amplitudes of individual subclass NMR signals directly correlate with the number of emitting particles. This NMR-based analysis of lipoprotein subclasses presents numerous advantages over alternative analytical methods: it is rapid, concurrently measures concentrations of all lipoprotein subclasses, requires no reagents, eliminates the need for physical separation, and involves minimal sample manipulation. Widely gaining clinical interest, this approach primarily focuses on assessing CVD risk. NMR-based lipidomic trials have unveiled that CAD's initiation and progression are related to modifications in many surface and core lipid components of HDL (atheroprotective) and non-HDL (atherogenic) lipoproteins beyond cholesterol content, as well as in fatty acids esterified in these lipids. Highlighting that an in-depth examination of the lipid composition of lipoproteins has the potential to uncover abnormal lipid-related pathways not detectable through conventional serum lipid parameters[100].

However, the association between lipidomics and lipid content in coronary atheromatous plaques, measured by NIRS, is an area of limited research. Previous studies have primarily focused on traditional lipid measurements and Lp(a), yielding either no associations or weak associations with plaque lipid content without analyzing a wider range of lipoprotein subfractions.

2. AIMS AND HYPOTHESES

2.1 General aims

The overall aim of this thesis was to expand the edge of knowledge on the effects of exercise training on coronary atheromatous plaques in patients with stable coronary artery disease, to potentially provide novel insight into preventive and therapeutic tools.

Specific aims and hypotheses:

1. To evaluate the effect of HIIT on coronary plaque composition in patients with stable CAD using NIRS.

We hypothesized that lipid content in coronary atheromatous plaques would be reduced after regular high-intensity interval training compared with usual care.

2. To assess the effect of HIIT on coronary plaque geometry in patients with stable CAD using IVUS.

We hypothesized that regular high-intensity interval training would induce plaque regression compared with usual care in patients with stable CAD.

3. To investigate the association between circulating lipoprotein subfractions assessed by nuclear magnetic resonance spectroscopy and lipid content in coronary atheromatous plaques assessed by NIRS in patients with stable CAD.

We hypothesized that there is an association between circulating lipoprotein subfractions, assessed through NMR spectroscopy, and lipid content within coronary atheromatous plaques, measured as $maxLCBI_{4mm}$ by NIRS in patients with stable CAD.

3. MATERIAL AND METHODS

3.1 Study design

This thesis is based upon an investigator-initiated, single-center, open, parallel RCT (Paper I and II) and a cross-sectional study based upon the baseline data from the RCT (Paper III). The study was conducted at the Clinic of Cardiology, St. Olavs University Hospital in Trondheim, Norway, between 2016 and 2019 (ClinicalTrials.gov: NCT02494947).

All three papers use data from the same cohort. Patients between 18 and 70 years old with stable CAD scheduled for coronary angiography were screened for inclusion. Only PCI-treated patients were included. Lipid-lowering therapy had to be used for at least six weeks before PCI. Table 2 summaries the inclusion and exclusion criteria

Table 2. Inclusion and exclusion criteria for enrolment in the studies in this thesis.

Inclusion criteria
✓ 18-70 years of age with stable coronary artery disease scheduled for coronary angiography
✓ PCI of flow-limiting lesions
✓ On stable lipid-lowering treatment for at least six weeks prior to inclusion
✓ Eligible to perform the exercise intervention
✓ Patient consent for participation
Exclusion criteria
✓ Previous coronary artery bypass surgery
✓ Known inflammatory disease other than atherosclerosis
✓ Planned surgery within the next six months
✓ Inability to comply with the study protocol due to any somatic disease, physical disability, or mental problems
✓ Inclusion in another randomized trial
✓ Already performing the physical exercise at a similar or higher level than the planned intervention

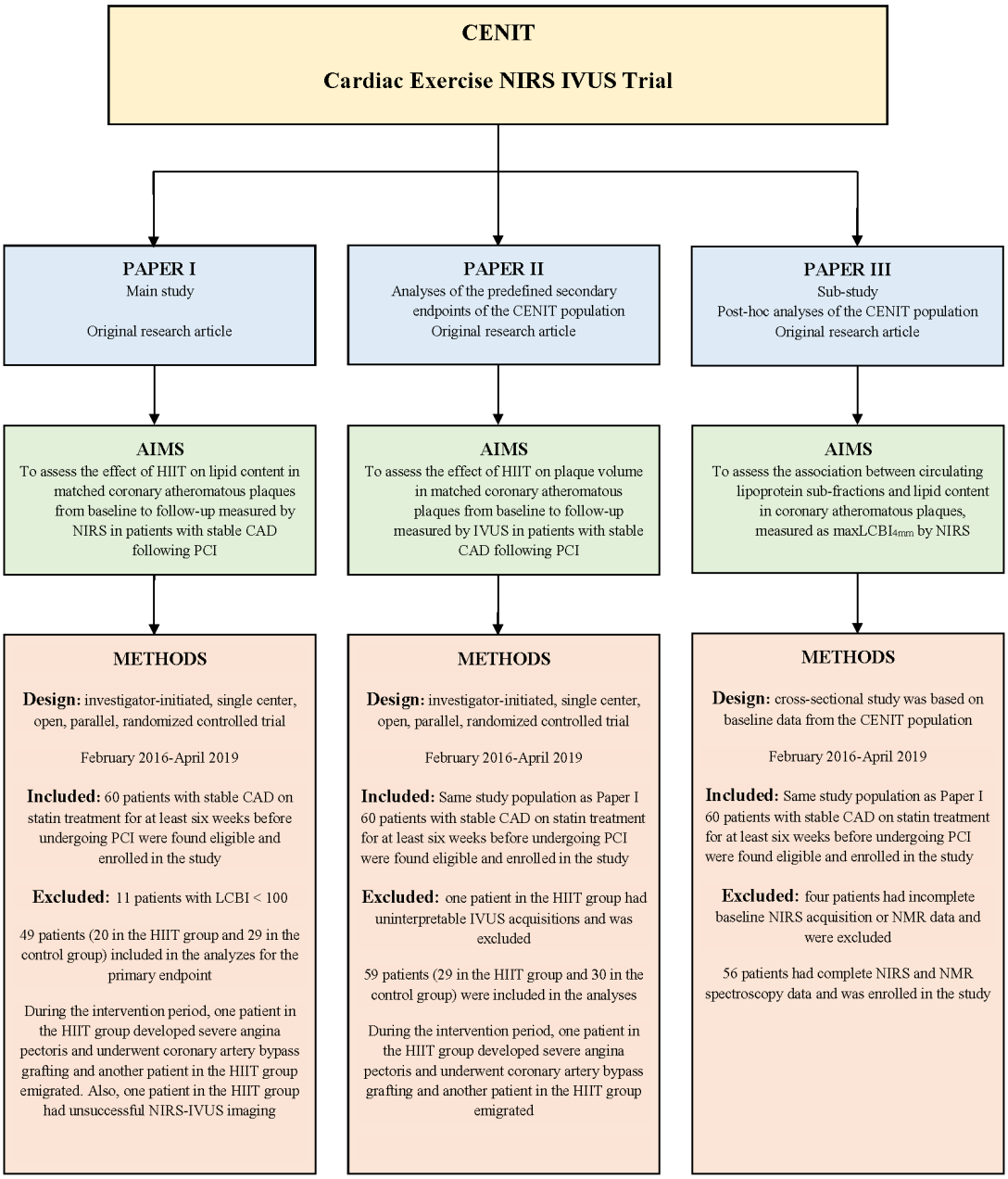

Figure 4. Overview of papers included in the theses.

Paper I*: CENIT (Impact of Cardiac Exercise Training on Lipid Content in Coronary Atheromatous Plaques Evaluated by Near-Infrared Spectroscopy) – A Randomized Trial.*

Sixty patients were initially enrolled and randomized in the CENIT study. Among those whose baseline assessments revealed no coronary plaques with a maximum lipid core burden index value exceeding 100 for any 4mm region (maxLCBI4mm), the lipid content was considered insufficient to predict changes in plaque composition with certainty. As a result, 11 patients were excluded from the study. All intravascular analyses were conducted offline by an external core laboratory; however, this data was only available once all patients had completed their follow-up assessments. Ultimately, 49 patients (20 in the HIIT group and 29 in the control group) were included and evaluated for the primary endpoint. Throughout the intervention period, a patient in the HIIT group experienced severe angina pectoris and required coronary artery bypass grafting. Another patient from the HIIT group emigrated. Furthermore, one HIIT group patient had unsuccessful NIRS-IVUS imaging during the follow-up evaluation. Consequently, follow-up NIRS-IVUS data were absent for these three patients.

Paper II: *High-intensity interval training induces beneficial effects on coronary atheromatous plaques – a randomized trial.*

The 60 patients who participated in the CENIT study (Paper I) were incorporated into this study. Unfortunately, one patient in the HIIT group had an unsuccessful intracoronary imaging and was not included in the subsequent analyses. Consequently, the final study cohort comprised 59 patients (29 in the HIIT group and 30 in the control group). One patient experienced severe angina pectoris during the follow-up period and required coronary artery bypass grafting, while another emigrated. As a result, 57 patients remained for the follow-up assessments.

Paper III: *The association between circulating lipoprotein subfractions and lipid content in coronary atheromatous plaques assessed by near-infrared spectroscopy.*

Out of the 60 patients enrolled in the RCT, this cross-sectional study includes 56 eligible patients with both evaluable NMR spectroscopy data and NIRS-derived $maxLCBI_{4mm}$ data.

3.2 Clinical variables and non-invasive data

We used the hospital's medical journal system (Doculive) to collect patients' medical history and medication information. Clinical variables such as hypertension, diabetes, prior history of CAD, and congestive heart failure were diagnosed in advance by the primary physician or during previous hospital visits.

3.2.1 Invasive procedures and imaging

Coronary angiography, PCI, and intracoronary imaging were performed according to standard techniques by experienced invasive cardiologists. Drug-eluting stents were used in all cases. In this study, the regions of interest were residual non-culprit atherosclerotic plaques typically of borderline angiographic significance. Following successful PCI and administration of intracoronary nitroglycerine, three-vessel intravascular imaging was performed when feasible by a 3.2 Fr-40 MHz TVC Insight Catheter using an automated pullback system (InfraReDx, TVC - MC8 model Imaging System™, Burlington, MA, USA). The NIRS-IVUS catheter was positioned as distally as possible in the coronary artery. Subsequently, the automated pullback was started at 0.5 mm/s (240 rotations/min) until the TVC catheter entered the guiding catheter. To ensure an adequate matching of the coronary segments, the distal starting points of the pullbacks were recorded angiographically to assist in registering the corresponding segments at follow-up. Standard transthoracic echocardiography was performed after the intravascular imaging.

3.2.2 Analyses of intravascular data

Angiograms and intravascular ultrasound data were interpreted offline at an independent core lab (KCRI, Krakow, Poland) without knowledge of patient characteristics or randomization allocation. Quantitative IVUS analyses were performed along the entire vessel length, and cross-sectional images were spaced 1.0 mm apart using CAAS IntraVascular Software 2.1 (Pie Medical Imaging B.V. Maastricht, Nederland). To ensure sufficient data quality, an initial review of all the intracoronary images was performed according to KCRI-GCL-SOP-30.010. Only diseased vessels with high acquisition quality and vessels including matching coronary segments were analyzed. The corresponding regions of interest at baseline and follow-up were identified using fiduciary points such as side branches and the implanted stent, as well as a comparison of angiographic records. Stented segments were excluded. Images were considered uninterpretable and rejected from further analysis if the corresponding block chemogram was black, indicating the absence of reliable data.

IVUS (Figure 5). In non-stented vessels, regions of interest (ROI) were identified as segment lesions where the atherosclerotic plaque caused a narrowing of the arterial lumen by at least 30% based on

cross-sectional area. The frame preceding the ROI served as the proximal reference, while the frame following the ROI served as the distal reference. In the case of stented vessels, separate ROIs were defined for segments located proximal or distal to the stent. Once the ROI was identified, the geometry of the vessel was examined meticulously at 1 mm intervals within the ROI.

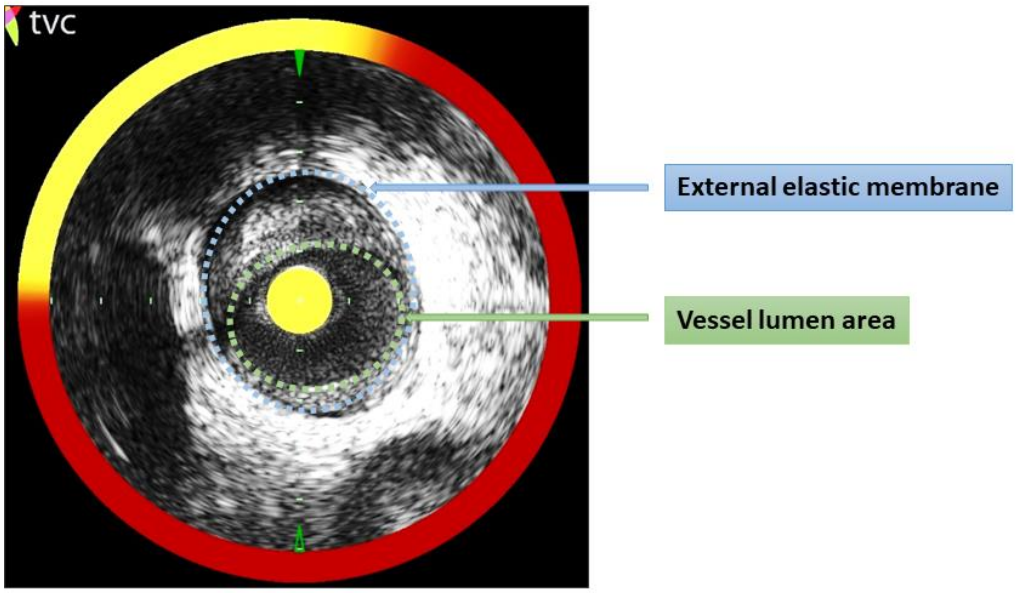

Figure 5. Intravascular imaging evaluation of a coronary lipid-rich plaque using NIRS-IVUS. The NIRS chemogram is located around the periphery of the greyscale IVUS image. Yellow indicates a high probability of lipids, while red represents no lipids. The IVUS image facilitates the assessment of the *atheroma area*= External Elastic Membrane $_{CSA}$- Vessel Lumen Area $_{CSA}$

In every cross-sectional image selected for analysis, the lumen cross-section area (lumen CSA) and external elastic membrane cross-section area (EEM CSA) leading edges were defined by planimetry. The total atheroma area was determined as the area between these leading edges. Percent atheroma volume (PAV) was calculated as the proportion of the vessel wall occupied by atherosclerotic plaque through the segment of interest using the following equation:

$$PAV = [\sum (EEM_{CSA} - Lumen_{CSA}) / \sum EEM_{CSA}] \cdot 100$$

The IVUS-derived, total atheroma volume (TAV) was calculated using the pullback speed during image acquisition. TAV was normalized to account for differences in segment lengths between patients:

$$TAV_{norm} = [\sum (EEM_{CSA} - Lumen_{CSA}) / \text{segment length}] \text{ x median segment length in the population}$$

The following parameters were calculated:

- Lesion length (mm)
- Minimal lumen area (mm^2)
- Minimal lumen diameter (mm)
- Vessel area (mm^2)
- Vessel diameter (mm)
- Plaque burden (%)
- Plaque volume (mm^3)

In the context of Paper II, the target vessel was identified as the vessel with the highest maximum plaque burden at the initial assessment. We then analyzed changes in plaque geometry measurements from the initial assessment to the follow-up evaluation within matched coronary segments. As depicted in Figure 6, we defined the minimum lumen area (MLA) as the smallest area at the center of the lumen within the region of interest. To evaluate remodeling, we employed the remodeling index (RI), which is calculated by dividing the cross-sectional area of the external elastic membrane (EEM CSA) at the MLA site by the proximal reference EEM CSA in the least diseased part of the target vessel. Positive remodeling was defined as $RI \geq 1.05$, and negative remodeling as $RI \leq 0.95$. Values in between were considered neutral (no remodeling).

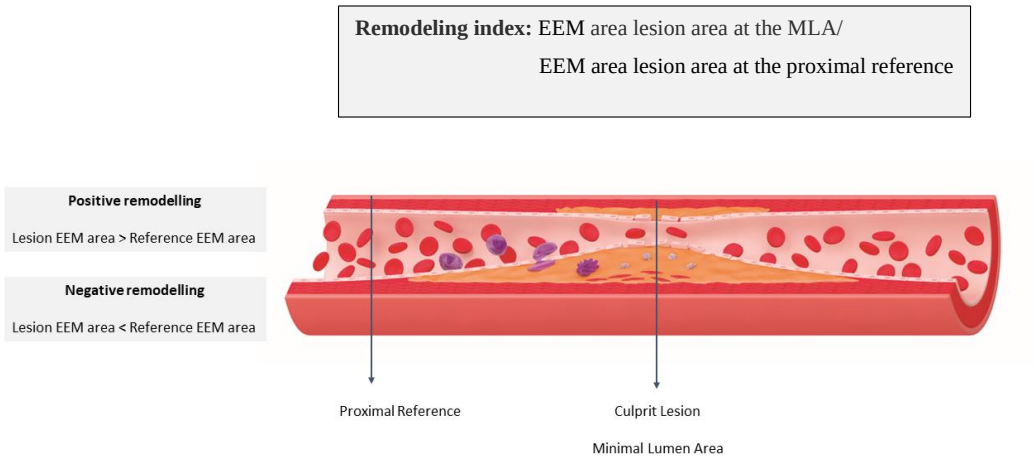

Figure 6. Illustration of a coronary artery with plaque accumulation in the vessel wall. Proximal reference: a normal vessel. Culprit lesion: Advanced atherosclerosis with narrowing lumen size due to plaque development. Minimal lumen area (MLA) is the narrowest luminal space of the coronary vessel. Remodeling index (RI): external elastic membrane area lesion (EEM) area at the MLA/external elastic membrane area lesion (EEM) area at the proximal reference.

LCBI (Figure 7). The estimation of lipid content within target lesions, Lipid Core Burden Index (LCBI), was assessed from NIRS data. The lipid-rich plaques are displayed on a chemogram where the X-axis shows the pullback position (1 pixel every 0.1 mm), while the Y-axis displays the circumferential position (1 pixel every 1 mm). Based on the chemogram, the $maxLCBI_{4mm}$ was measured automatically by the NIRS software in every artery segment, irrespective of where it was in the coronary artery tree. In Paper I, the plaque with the highest $maxLCBI_{4mm}$ at baseline was defined as the target lesion, and the change in lipid content in matched coronary segments from baseline to follow-up was studied. To detect lipid content alterations, only coronary atheromatous plaques with a $maxLCBI_{4mm} > 100$ at baseline were included in the analyses. In Paper I and III, the plaque with the highest $maxLCBI_{4mm}$ at baseline was defined as the target lesion and used in outcome analyses.

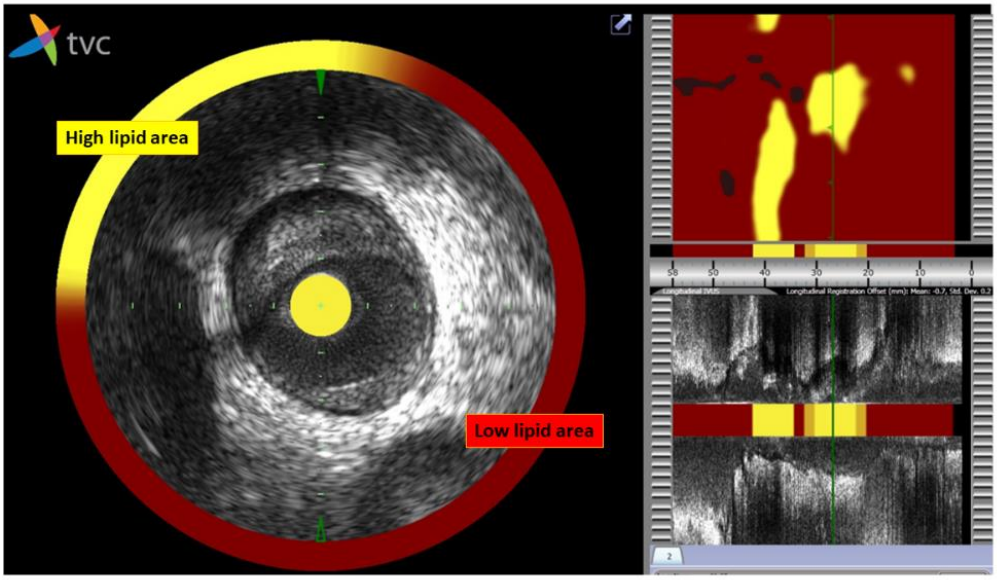

Figure 7. Illustration of intravascular combined NIRS-IVUS imaging. To the left, a cross-sectional greyscale IVUS image reveals a moderate plaque with a color-coded circumflex illustrating lipid accumulation within the plaque. Yellow indicates a high probability of lipids, while red represents no lipids. To the right, a longitudinal image with the NIRS-derived chemogram illustrates the $\text{maxLCBI}_{4\text{mm}}$ segment across the entire lesion.

3.2.3 Biochemical measurements

Fasting blood samples were drawn the day after PCI. Following standard in-hospital procedures, fasting venous blood samples were obtained and drawn into endotoxin-free blood collection tubes with EDTA as anti-coagulants (plasma) and with no additives (serum). Hemoglobin, creatinine, glycated hemoglobin A1c (HbA1c), total cholesterol, HDL cholesterol (HDL-C), LDL-C, triglycerides, Apo-A1, Apo-B, and Lp(a) were analyzed in blood samples using standard in-hospital procedures at the Department of Medical Biochemistry, St. Olavs Hospital. The tubes for biobanking were immediately placed on melted ice and centrifuged (Rotina 420R, Hettich zentrifugen) within 1 hour, at 3000 × *g* for 10 minutes at room temperature (20°C). Immediately following centrifugation, the sample was aliquoted into microfuge tubes, marked, and stored in a local biobank at - 80°C until NMR spectroscopy analysis.

3.2.4 Lipoprotein subfractions analyzed by NMR spectroscopy

NMR lipidomic analyses were undertaken at the MR Core Facility, NTNU. Using Bruker Avance III Ultrashield Plus 600 MHz spectrometer (Bruker BioSpin, GmBH, Rheinstetten, Germany) density, size, and particle number of the different lipoprotein subfractions were quantified based on H-NMR spectroscopy. Mixed thawed serum (150 μl) and buffer (150 μl, 20 % D_2O with 0.075 M Na_2HPO_4, 6 mM NaN_3, 4.6 mM trimethylsilyl-propanoic acid, pH 7.4) were transferred to 3mm NMR tubes, and SampleJet with Icon-NMR on Topspin 3.1 (Bruker BioSpin) fully automated further procedures. 1D 1H Nuclear Overhauser effect spectroscopy (NOESY) and Carr-Purcell-Meiboom-Gill (CPMG) spectra with water pre-saturation were obtained at 310 K. The spectra were Fourier transformed to 128 K after 0.3 Hz exponential line broadening. Lipoproteins were scanned by size, density, and lipid content, and the lipid profile was provided by Bruker IVDr Lipoprotein Subclass Analysis (B.I.LISA^{TM})[106]. This method quantifies serum concentrations of cholesterol, free cholesterol, phospholipids, and triglycerides within various lipoproteins (HDL, VLDL, IDL, and LDL) and their density-based subfractions (HDL 1-4, VLDL 1-5, and LDL 1-6, increasing in density). Additionally, Apo-A1, Apo-A2, and Apo-B were measured in both serum and within the subfractions. LDL-C/HDL-C and Apo-B/Apo-A1 were calculated. The quantity of lipoprotein particles (particle numbers) for VLDL, IDL, LDL, and LDL-1-6 were determined. The NMR lipidomic analysis generates a dataset of 112 variables for each sample.

3.3 Hospital discharge

The patients were discharged from the hospital the day after PCI. Guideline-directed medical therapy was administered to all patients. However, no adjustments were made for lipid-lowering treatment throughout the study. At discharge, all patients were given general advice to maintain a healthy lifestyle, including diet, smoking cessation, use of prescribed medication, achieving a blood pressure <140/90 mmHg, HbA1c values < 53 mmol/mol (7%), and performing regular exercise. They were also encouraged to participate in local CR programs and heart schools.

3.4 Cardiopulmonary exercise testing

An individualized cardiopulmonary exercise test (CPET) was performed two weeks following PCI and before randomization to determine exercise tolerance, VO_{2peak}, and HR_{peak}. Data from the 12-lead electrocardiogram, blood pressure-, and gas exchange measurements were continuously recorded using a direct ergospirometry system (Vyntus CPX, Erich Jaeger GmbH, Hoechberg, Germany). The test was performed on a treadmill, walking, or running, depending on the patient's fitness level. After a brief 10-15 min warm-up, the test started at a workload derived from the warm-up settings. Using an individualized protocol, the workload was increased by speed and/or an incremental inclination every minute until exhaustion or flattening of the oxygen uptake curve ($\Delta\ VO_{2max} < 2\ mL \cdot kg^{-1} \cdot min^{-1}$) despite

increasing workload combined with a respiratory exchange ratio (RER) above 1.05 (VO_{2max}). The protocol lasted eight to twelve minutes. VO_{2peak} was the average of the highest 30-s oxygen uptake value achieved during testing.

3.5 Randomization and treatment

After completing baseline acquisitions and the CPET, block randomization was performed by a web-based system developed and administered by the Unit of Applied Clinical Research, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway. Patients were randomly allocated to twice-weekly sessions of supervised HIIT at 85% to 95% of HR_{peak} versus contemporary standards of care for six months with no protocol-based follow-up until the end of the study period.

3.6 Intervention

The intervention group joined supervised endurance training performed as walking or running on a treadmill or bicycling following the HIIT principle twice weekly for six months. Each session started with a ten-minute warm-up at moderate intensity (60 % to 70 % of HR_{peak}), followed by 4 x 4-minute intervals with intensity at 85 % to 95 % of HR_{peak} with three minutes of active recovery at a moderate intensity between intervals ending with a five-minute cool-down period. Additionally, patients were encouraged to perform home-based endurance training with bouts of intervals, and any activity mode involving dynamic work with large muscle groups was recommended. To ensure adequate training intensity and increase adherence, the patients in the HIIT group were encouraged to use a wearable device for HR and activity tracking (Mio Global PAI, Toronto, Canada). Only the HIIT group was equipped with this device. The rationale behind its use was to increase the patient's PA level and motivation for performing home-based endurance training. The device also displayed heart rate; thus, direct feedback regarding training zones was available. We did not track the activity in the control group in order to avoid enhancing the Hawthorne effect. The control group was encouraged to practice regular PA of moderate intensity according to current guidelines without receiving any further supervision.

3.7 Follow-up acquisitions

After six months, the patients were readmitted for follow-up CPET, echocardiography, blood sampling, and intravascular imaging.

3.8 Endpoints

Paper I

The primary endpoint in Paper I was $\Delta maxLCBI_{4mm}$. In this study, the between-group difference in the mean change in lipid content from baseline to follow-up in matched coronary plaques was measured by NIRS. Secondary outcomes were between-group differences in alterations in VO_{2peak}, BMI, waist circumference, blood lipids including total-cholesterol, LDL-, and HDL-cholesterol, triglycerides, apolipoprotein A, apolipoprotein B, and glycosylated hemoglobin A1c (HbA1c). The within-group changes for these variables were also analyzed.

Paper II

In Paper II, we present the results from the analyses of the predefined secondary endpoints assessing plaque geometry with IVUS. The efficacy outcomes were the between-group differences in mean changes in PAV and TAV_{norm} in matched coronary plaques. Other outcome measures were the between-group differences in mean change in remodeling index, minimal lumen area, VO_{2peak}, BMI, waist circumference, blood lipids including total-, LDL-, and HDL-cholesterol, triglycerides, apolipoprotein A, apolipoprotein B, and glycosylated hemoglobin A1c (HbA1c). Additionally, the within-group changes during the study period were also evaluated.

Paper III

In Paper III, the primary endpoint was the association between circulating lipoprotein subfractions and lipid content in coronary atheromatous plaques measured by NIRS.

Table 3 summarizes efficacy endpoints, intravascular imaging and statistical methods used in the three papers.

Table 3. Overview of the efficacy endpoints, main methods, and statistical methods used in the papers.

	Endpoints	Method	Statistical method
Paper I	$\Delta maxLCBI_{4mm}$	NIRS	Linear Mixed Model
Paper II	ΔPAV % ΔTAV_{norm} mm^3	IVUS	Linear Mixed Model
Paper III	Association between $\Delta maxLCBI_{4mm}$ and lipoprotein subfractions	NIRS NMR spectroscopy	Penalized linear regression analysis LASSO

3.9 Statistics

3.9.1 Power calculations

In the planning of the CENIT study, there were no published data on the effect of physical exercise on $maxLCBI_{4mm}$. In a previous IVUS study from our research group, we demonstrated a reduction in necrotic core for 36 PCI-treated patients undertaking 12 weeks of HIIT or exercise at moderate intensity with no in-between group difference[107]. Based on this experience, we designed the CENIT study with an increase in sample size (60 patients) and prolonged the intervention period (six months) to increase the statistical power concerning the primary endpoint.

3.9.2 Statistical analyses

Statistical analyses were performed using SPSS (Armonk, NY: IBM Corp, version 26.0 in papers I and II, and version 27.0 in paper III), R (version 4.0.2 in paper III), and Microsoft Office Excel 2016. Continuous variables are presented as means with standard deviations, medians with interquartile ranges (IQR), and categorical data as frequencies with percentages. Normally distributed continuous variables were compared using the Student t-test and categorical variables by the Chi-square or Fisher's exact test. P-values <0.05 were considered statistically significant, and confidence intervals (CIs) were presented at the 95% level. The normality of residuals was checked by visual inspection of QQ plots. No formal adjustment for multiple testing was performed.

In Paper I and II, the study endpoints were tested using linear mixed models (LMM) with time and intervention group (HIIT or control) as fixed effects variables. The dependence of observations within individuals was handled by including individual-specific random intercepts[108]. Due to randomization, mean outcomes at baseline were in the models constrained to be equal between the groups. Similar models were used for the other outcome measurements. All principal analyses were done in the intention-to-treat population, consisting of all randomized patients regardless of treatment. In paper I, we used the Spearman's correlation coefficient to study the relationship between the change in VO_{2peak} (ΔVO_{2peak}) and $\Delta maxLCBI_{4mm}$ from baseline to follow-up.

In paper III, lipid variables were assessed for normality by the Shapiro-Wilk test and visual inspection of normal QQ plots. Pearson or Spearman correlations were used to evaluate the correlation between each lipid variable and $maxLCBI_{4mm}$. To estimate the effective number of independent tests for multiple testing corrections, principal component analysis (PCA) was used. Nine principal components explained >95 % of the variance, and the corrected threshold for assessing statistical significance was 0.05/9= 0.005 ($p<.005$). The rationale for this method has been described previously, and this method has been applied in several metabolic profile studies [109,110]. In cases where there are large multivariable data sets containing numerous variables larger than the sample numbers, a standard linear model performs poorly. For this reason, we used the penalized regression method: Least Absolute Shrinkage and Selection Operator (LASSO), glmnet package[111] in R, when analyzing the association between the lipidomics and the NIRS data. We determined the degree of shrinkage by 10 x 10-fold cross-validation, minimizing the mean square error. This shrinkage/regularization method aims to reduce the complexity by imposing a penalty, forcing some of the coefficient estimates, with a trivial contribution to the model, to be close or equal to zero. The method can simultaneously perform parameter estimation and variable selection, as some variables are minimized to precisely zero.

To evaluate the uncertainty of the estimated lasso coefficients, we employed bootstrapping by repeating the fitting procedure for 1000 bootstrap samples. This approach allowed us to assess the variability in the coefficients. For each variable, we determined the proportion of bootstrap samples in which the coefficient was not set to zero in the estimated model. This proportion represented the uncertainty associated with each variable. We conducted the model fitting on two different sets of predictors. The first set included lipoprotein subfractions and Lp(a), and it consisted of 107 samples. The second set incorporated both lipoprotein subfractions and established risk factors for CVD, and it comprised 121 samples. These risk factors include age, BMI, smoking status, diabetes mellitus (medically treated), hypertension (medically treated), hyperlipidemia, heredity of premature CVD, previous CVD, and lipid profile, including total cholesterol, LDL-C, HDL-C, LDL-C/HDL-C, Apo-B/Apo-A1, and triglycerides. All variables were continuous, except Lp(a) (above/beneath 30 mg/dL), smoking (yes/no), diabetes

(yes/no), hypertension (yes/no), hyperlipidemia (yes/no), previous CVD (yes/no), and heredity of CVD (yes/no).

3.10 Ethics

The Regional Committee for Medical Research Ethics (REK, 2015/210), the research committee at the Clinic Cardiology at St. Olavs hospital, and the Institute for Circulation and Medical Imaging, NTNU, approved the research project. All patient information was stored and handled with high levels of security according to laws and regulations. The study was registered at clinicaltrials.gov (NCT02494947) and performed according to the Declaration of Helsinki and Good Clinical Practice (GCP). Written informed consent was obtained from all participants.

4. RESULTS

Table 4 illustrates the main results from the 3 different papers

Table 4. Baseline Clinical Characteristics of the Randomized Population

	Paper I		**Paper II**		**Paper III**
	HIIT(n=20)	Usual Care(n=29)	HIIT(n=29)	Usual Care(n=30)	(n=56)
Characteristics					
Age, years	57.6 ± 6.2	58.4 ± 7.4	57.3 ± 6.8	58.7 ± 7.4	57.8 ± 7.1
N, males/females	19/1	26/3	27/2	27/3	53/3
BMI, kg/m^2	29.1 ± 4.4	29.2 ± 3.6	28.9 ± 4.0	29.1 ± 3.5	29.0 ± 3.7
VO_{2peak}, $mL \cdot kg^{-1} \cdot min^{-1}$	32.3 ± 5.9	29.1 ± 6.2	32.6 ± 5.8	29.0 ± 6.1	
Medical history, *n* (%)					
Hypertension	9 (45)	15 (52)	16 (55)	16 (53)	30 (54)
Diabetes	1 (5)	5 (17)	2 (7)	5 (17)	7 (12)
Smoking currently	1 (5)	4 (14)	1 (4)	4 (13)	5 (9)
Prior history of CAD	11 (55)	15 (52)	11 (38)	13 (43)	27 (48)
Congestive heart failure	1 (5)	2 (7)	1 (4)	2 (7)	2 (4)
Medication at baseline					
Dual antiplatelet	20 (100)	29 (100)	29 (100)	30 (100)	56 (100)
Statins	20 (100)	29 (100)	29 (100)	30 (100)	56 (100)
Statin + Ezetimibe	1 (5)	3 (10)	2 (7)	3 (10)	2 (4)
Beta-blockers	8 (40)	10 (35)	10 (35)	11 (37)	20 (36)
RAAS-blockers	9 (45)	14 (48)	16 (55)	14 (47)	29 (52)

Values are presented as mean ± standard deviations for continuous variables and frequencies (%) for categorical variables. RAAS-blockers; Angiotensin-converting enzymes inhibitors/angiotensin II receptor antagonist

Paper I

Forty-nine patients (20 in the HIIT group and 29 in the control group) were included and analyzed for the primary endpoint. During the intervention period, one patient in the HIIT group developed severe angina pectoris and underwent coronary artery bypass grafting. Another patient in the HIIT group emigrated. In addition, one patient in the HIIT group had unsuccessful NIRS-IVUS imaging at follow-up. Thus, there was no follow-up NIRS-IVUS data from these three patients. One patient in the control group suffered a minor stroke at the end of the follow-up period. Otherwise, there were no adverse events during the study. In particular, there were no adverse events related to the invasive procedures. Baseline patient characteristics were similar between the two groups (Table 4). The patients were middle-aged, with only 8 % women. Traditional cardiovascular risk factors, including previous or current smoking, were prevalent. At baseline, the $maxLCBI_{4mm}$ levels were well balanced. When comparing $maxLCBI_{4mm}$ between different arteries in the same patient, we observed that lipid content in atheromatous plaques changed in both directions in 16 patients at follow-up. No consistent pattern concerning changes in $maxLCBI_{4mm}$ based on the anatomical origin of the coronary artery was observed.

Key findings

- After six months, there was no evidence of differences between the two groups when comparing the change in plaque lipid content ($\Delta maxLCBI_{4mm}$: -1.2 (-65.8 to 63.4) p =0.97). As highlighted in Figure 8, both groups demonstrated similar reductions (13%–14%) in average $\Delta maxLCBI_{4mm}$with a statistically significant reduction only in the control group.

- A significant improvement in CRF was demonstrated after six months of supervised HIIT compared to standard care (p= 0.03). VO_{2peak} increased by 3.9 mL $\cdot$ $kg^{-1}\cdot$ min^{-1} after HIIT ($p<0.001$), while the control group showed a non-significant increase of 1 mL $\cdot$ kg^{-1} $\cdot$ min^{-1}.

- BMI and waist circumference were more reduced after HIIT than usual care. Both groups demonstrated a modest but significant increase in HDL-cholesterol, 0.2 vs. 0.1 for the HIIT and the control groups, respectively, with a significant between-group change (p=0.04). An increase in Apo-A1 was observed in both groups while there were no changes in LDL, Apo-B or HbA1c levels.

- When combining the two groups, a secondary analysis showed a moderate negative correlation between increasing fitness and change in $maxLCBI_{4mm}$ (Spearman's rho = −0.44, p=0.009). Among patients with an increase in VO_{2peak} above 1 MET (3.5 mL · kg^{-1}· min^{-1}), $maxLCBI_{4mm}$ was, on average, reduced by 142 (range -8 to -262), whereas the change was -3.2 (range 154 to -255) among those with an increased VO_{2peak} below 1 MET. This correlation is illustrated in Figure 9. Among the 17 patients with an increased VO_{2peak} below 1 MET, nine had increased, and eight had decreased $maxLCBI_{4mm}$.

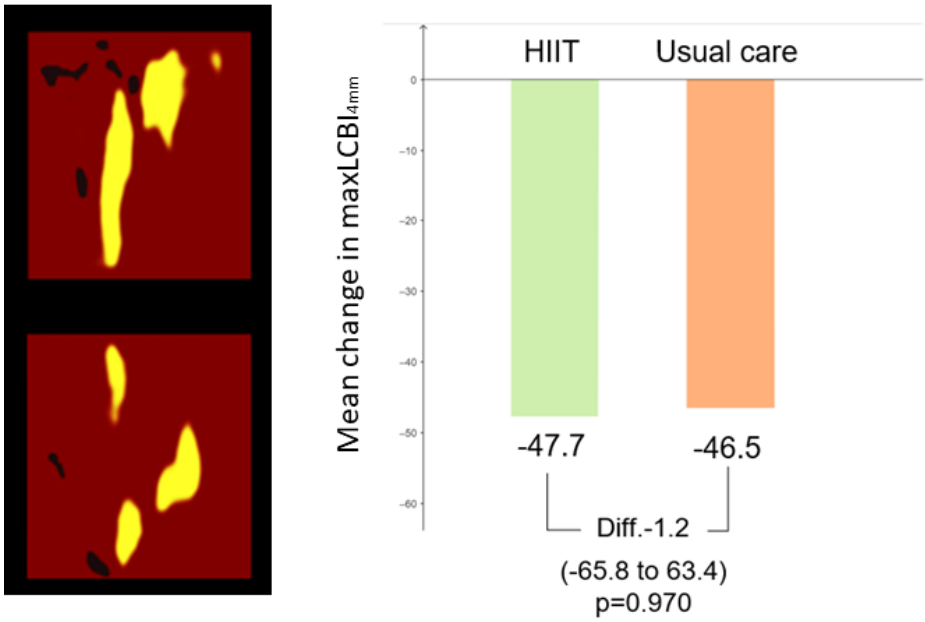

Figure 8. NIRS chemogram of a residual lipid-rich plaque at baseline (left upper) and at six months follow-up (left lower). At six months, no statistically significant differences were observed between the two groups regarding the change in plaque lipid content (right). Lipid content was reduced in both groups, with a statistically significant reduction only in the control group.

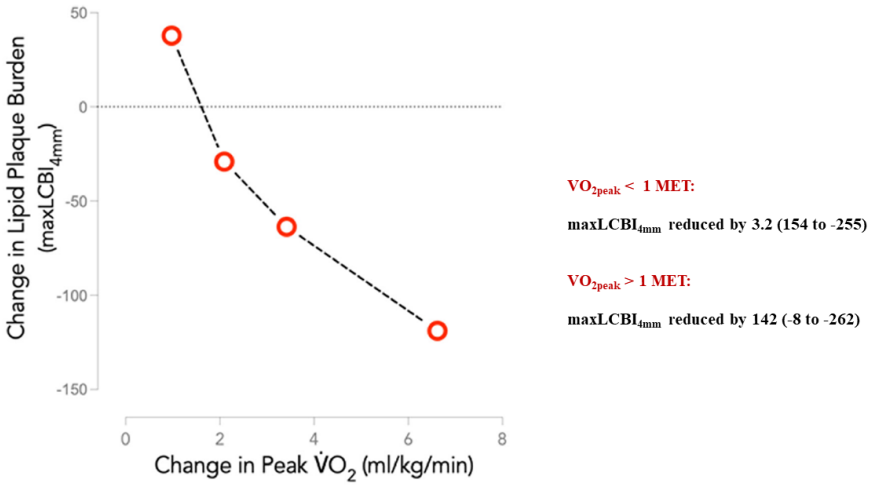

Figure 9. Influence of improved CRF on lipid content in coronary plaques.
Results from secondary analyses of combined groups regardless of the type of exercise.
Reprinted from Guseh et al, *Journal of the American Heart Association*, 2022.

Table 5. Baseline and follow-up data from intravascular imaging of the randomized population

Paper I	**Exercise group HIIT(n=20)**		**Control group (n=29)**	
	Baseline	Follow-up	Baseline	Follow-up
MaxLCBI$_{4mm}$	357 ± 136	306 ± 150	336 ± 157	292 ± 186
Paper II	**Exercise group HIIT(n=29)**		**Control group (n=30)**	
Max PAV (%)	66.3 ± 12.1	65.3 ± 10.2	67.2 ± 8.4	68.6 ± 9.6
Average PAV (%)	49.5 ± 9.4	48.3 ± 8.4	48.6 ± 6.1	48.9 ± 6.0
TAV$_{norm}$ (mm^3)	162.7 ± 60.5	154.9 ± 57.2	179.4 ± 61.4	182.1 ± 63.3
Atheroma volume (mm^3)	187.9 ± 179.7	173.6 ± 162.8	229.6 ± 124.5	233.2 ± 130.3
Remodeling index	0.9 ± 0.2	0.8 ± 0.2	0.9 ± 0.1	0.9 ± 0.2
MLA (mm^2)	4.3 ± 1.6	4.3 ± 1.6	4.7 ± 1.3	4.4 ± 1.4

Paper II

The study endpoints in Paper II were changes in percent atheroma volume (PAV) and total atheroma volume (TAV) normalized for segment length (TAV_{norm}) at a six-month follow-up. Baseline clinical features, laboratory measures, and lesion characteristics of the randomized population were well-matched and similar in most respects (table 4). In particular, coronary atheromatous lesions in the target artery were similar between the two groups at baseline.

Key findings

- The change in average PAV for matched coronary segments from baseline to follow-up showed a significant between-group difference. In the HIIT group, a significant plaque regression was observed, while not in the control group (Figure 10). Change in mean normalized total atheroma volume (TAV_{norm}) was also significantly different between the groups and there was a reduction in the remodeling index in the HIIT group while not in the control group. There were no significant differences or changes in MLA during the study (Table 6).

- A significant improvement in cardiorespiratory fitness was demonstrated after six months of supervised HIIT compared to standard care ($p= 0.04$). VO_{2peak} increased by 2.7 mL $\cdot$ $kg^{-1}\cdot$ min^{-1} after HIIT ($p<0.001$), compared to a non-significant increase of 1 mL $\cdot$ $kg^{-1}\cdot$ min^{-1} in the control group.

- BMI and waist circumference were significantly reduced after HIIT, while not in the control group. Both groups observed a modest increase for HDL-cholesterol and Apo-A1, with no evidence of between-group differences. There were no significant differences or changes at follow-up in other biochemical variables.

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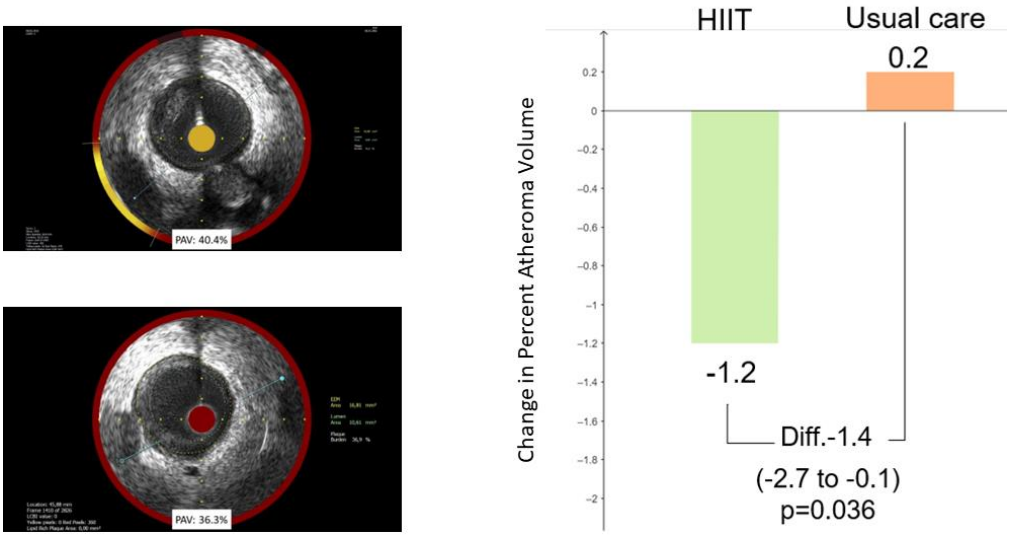

Figure 10. Cross-sectional NIRS-IVUS images of a residual lipid-rich plaque at baseline (left upper) and at six months follow-up (left lower) depicting notable plaque regression following HIIT. Statistically significant distinctions in the alteration of percent atheroma volume (PAV) were observed between the two cohorts (right). Specifically, the HIIT group demonstrated a change of -1.2% while the usual care group exhibited a change 0.2%, p =0.036.

Table 6. Selected Outcomes from intravascular imaging variables from Paper II

	Change Baseline to Follow-up HIIT Group (n=29)	Change Baseline to Follow-up Control Group (n=30)	Group differences HIIT vs. Control Group
TAV_{norm} (mm^3)	-9.0 (-14.7 to -3.4) *p=0.002*	3.0 (-2.4 to 8.4) *p=0.268*	-12.0 (-19.9 to -4.2) *p=0.003*
MLA (mm^2)	-0.1 (-0.4 to 0.2) *p=0.454*	-0.2 (-0.5 to 0.1) *p=0.152*	0.1 (-0.3 to 0.5) *p=0.646*
Remodeling index	-0.1 (-0.1 to 0.0) *p<0.001*	0.0 (-0.1 to 0.0) *p=0.633*	-0.1(-0.1 to 0.0) *p=0.026*

Table 6. presents selected outcomes derived from IVUS variables. Continuous variables are presented as mean ± SD for. The outcomes demonstrate the treatment effect as time x group interaction, along with 95% confidence intervals and p-values for HIIT group compared to the control group. Abbreviations: TAV, Total atheroma volume; MLA, Minimal lumen area.

Paper III

This study aimed to investigate the association between circulating lipoprotein subfractions and lipid content in coronary atheromatous plaques. Out of the initial 60 patients enrolled in the CENIT study, 56 met the criteria for inclusion and had complete NMR spectroscopy and NIRS-derived maxLCBI$_{4mm}$ data. This cross-sectional study revealed modest evidence of an association between lipoprotein subfractions and maxLCBI$_{4mm}$. Based on the resampled datasets, we identified two lipoproteins with potential as predictors: Lp(a) and free cholesterol in the smallest HDL subfractions (HDL-4). Lp(a) was present in 78.1% of the resampled datasets. Patients with elevated Lp(a) levels (>30 mg · dL^{-1}) had an estimated average of 57.0 units higher maxLCBI$_{4mm}$ values compared to patients with Lp(a) levels below 30 mg · dL^{-1}. Free cholesterol in HDL-4 was included in 74.3% of the resampled datasets, and there was an estimated average increase of 36.5 units in maxLCBI$_{4mm}$ for every unit increase (mg · dL^{-1}) These findings indicate a potential association between these lipoproteins and the lipid content of atheromatous plaques.

Key findings

- The correlation analysis between maxLCBI$_{4mm}$ and lipid variables showed a range of values from -0.293 to 0.196 (as measured by the Spearman's correlation coefficient). However, after adjusting for multiple testing, none of these correlations were statistically significant. Cholesterol in the smallest VLDL subfractions (VLDL-5) exhibited the strongest correlation with maxLCBI$_{4mm}$, with a Spearman's correlation coefficient of -0.293 (p= 0.028). Despite this relatively stronger correlation, the statistical significance did not withstand adjustment for multiple testing.

- In the multivariable analysis, Lp(a) and free cholesterol in the smallest HDL subfractions (HDL-4) showed the strongest associations with maxLCBI4mm were. These lipoproteins had the highest percentage presence in the resampled datasets, indicating their potential predictive value for maxLCBI$_{4mm.}$

- After including established cardiovascular risk factors in the regression model, none of the lipoproteins were identified as potential predictors of maxLCBI$_{4mm}$. The influence of these lipoproteins on maxLCBI$_{4mm}$ seems to be attenuated or explained by the presence of other established cardiovascular risk factors in the model. However, there is limited knowledge of the influence of traditional cardiovascular risk factors on coronary lipid content.

5. DISCUSSION

This thesis presents data from a randomized clinical trial using near-infrared spectroscopy and intravascular ultrasound to evaluate the potential anti-atherogenic effects of high-intensity interval training in patients with established coronary artery disease (Papers I and II). Additionally, in a cross-sectional study based on the baseline data from the CENIT population, we aimed to investigate the association between circulating lipoprotein subfractions and lipid content in coronary plaques (Paper III).

The main findings were:

1. Six months of supervised HIIT did not modify plaque composition to a greater extent than standard care. Both groups exhibited de-lipidification with a numerical reduction in coronary lipid content. However, this change was statistically significant only in the control group. When combining the two groups, a secondary analysis showed a moderate correlation between increasing fitness and decreasing lipid content, indicating potential benefits of improved fitness on coronary plaque characteristics (Paper I).

2. HIIT induced plaque regression by reducing atheroma volume. The patients in the HIIT group demonstrated a more substantial reduction in percent atheroma volume (PAV), total atheroma volume (TAV), and remodeling index than the unsupervised control group (Paper II).

3. HIIT improved cardiovascular health by significantly increasing cardiopulmonary fitness. Waist circumference and body mass index were reduced more substantially after HIIT (Paper I and II).

4. There were no correlations between lipoprotein subfractions assessed by nuclear magnetic resonance spectroscopy and coronary plaque lipid content ($maxLCBI_{4mm}$) assessed by NIRS (Paper III).

5. In an exploratory analysis, Lp(a) and free cholesterol in the smallest HDL subfractions (HDL-4) were the lipoproteins most strongly associated with coronary plaque lipid content ($maxLCBI_{4mm}$). However, after including established cardiovascular risk factors in the regression model, none of the lipoproteins appeared as potential predictors of $maxLCBI_{4mm}$.

Acute coronary syndromes are often caused by disrupted and thrombotic, vulnerable plaques. Even though lesions responsible for unexpected events often appear to have mild angiographic findings, most of them exhibit phenotypic morphologic characteristics such as thin-cap fibroatheromas, a large plaque burden, high lipid content, and a reduced luminal area, or a combination of these features, as determined

by intravascular imaging[13]. Despite the advancements in percutaneous coronary intervention and pharmacological treatments that have improved prognosis for these patients, a significant proportion continue to experience recurrent cardiovascular events[23] and further add-on treatment options are warranted.

The association between PA and CAD has been known for decades and among patients with established CAD, there is evidence that exercise attenuates and halts the atherosclerotic process. An updated 2021 Cochrane Review confirms the significant benefits of exercise-based CR in providing essential advantages for CAD patients, impacting disease progression, survival, and quality of life[72]. These benefits are thought to be secondary to modulation of signaling pathways involved in cardiac remodeling encompassing structural, electrical, functional, and vascular changes in addition to the direct effects on the cardiac risk profile. Discharging patients with cardioprotective medications, initiating lifestyle management, and referring them to CR are strongly recommended as Class 1A in current guidelines[27]. Although it has been hypothesized that exercise training may have anti-atherogenic effects, strong evidence for this has not been demonstrated in humans. So far, only a few studies have used intracoronary imaging when assessing the effects of exercise.

5.1 Exercise training and the effect on coronary plaque

In 1990, Ornish et al. were the first to demonstrate an exercise-induced plaque regression after one year of moderate exercise associated with a low-fat Mediterranean diet, generating a 3.1% regression in coronary stenosis by Quantitative Coronary Angiography without using lipid-lowering drugs [94]. Since then, only a few exercise studies have tested the effect on coronary plaque regression, and overall, they have indicated that exercise can slow the advancement or induce modest regressions of coronary lesions. Two crucial factors affecting the impact of exercise on the heart, including coronary lesions, are the dose and the modality of the exercise training. In 1993, Hambrecht et al. designed a study to define the effect of different leisure time physical activity levels on CRF and the progression of coronary atherosclerotic lesions assessed by Quantitative Coronary Angiography in unselected patients with CAD[112]. This study found that measurable improvement in CRF required ~1,400 kcal/week of leisure time physical activity; higher workloads were necessary to halt the progression of coronary atherosclerotic lesions (1,533 ± 122 kcal/week), whereas regression of coronary lesions was observed only in patients expending an average of 2,200 kcal/week in leisure time physical activity, amounting to ~5 to 6 h/week of regular physical exercise. These observations support one of the current understandings that a critical exercise volume must be achieved to induce substantial effects on coronary plaques. In 2010, Sixt et al.[97] demonstrated an improvement in coronary endothelial function after a multifactorial six-month intervention program combining a hypocaloric diet with exercise in patients with type 2 diabetes. However, by IVUS, no significant effect on coronary plaque burden was found.

The first plaque regression studies were designed using aerobic continuous moderate-intensity exercise. However, in recent years, HIIT has been explored to an increasing extent. The rationale is the hypothesis that this exercise modality induces higher endothelial shear stress during repeated exercise peaks at maximal intensity, which could be more effective in eliciting plaque regression. In a previous study from our research group, Madssen et al.[98] compared three months of HIIT vs. continuous aerobic training and found that both exercise modalities induced a significant reduction in coronary necrotic core with no between-group differences. These findings were reproduced in a small study by Nishitani-Yokoyama et al.[99], demonstrating a correlation between exercise volume and a reduction in plaque and lipid volume, but no significant difference in the change of plaque regression or components between the intensive and standard CR groups.

The current thesis, derived from the CENIT trial, marks a methodological progress with an expanded sample size and an extended intervention period. Within this trial, comprehensive multi-vessel intracoronary NIRS-IVUS imaging was employed, providing valuable insights into the overall atherosclerosis burden, encompassing a thorough assessment of the coronary plaque transformations from the initial assessment to the six-month follow-up. Overall, this thesis contributes to the hypothesis that exercise may confer some cardiovascular benefits by improving plaque composition and inducing further plaque regression.

5.2 Lipid-lowering therapy and the effect on coronary plaque

It is worth noting that all patients in the CENIT study were already receiving stable statin therapy, and some were additionally prescribed combination therapy with ezetimibe at the time of their inclusion in the study. From a scientific point of view, the ideal protocol to assess the effect of exercise on lipid content in coronary atheromatous plaques would be to include statin naïve patients with no lipid-lowering therapy during the intervention period. However, lipid-lowering therapy is a first-line treatment in the primary[4] and secondary prevention[27] of CAD, and the clinical evidence on the efficacy of lipid-lowering therapy in patients with coronary artery disease is unequivocally established[113]. Incremental LDL-C lowering with statins, ezetimibe, alirocumab, and evolocumab have all improved cardiovascular outcomes in the CTT, IMPROVE-IT, ODYSSEY LONG TERM, and the FOURIER studies. Besides their undisputable lowering effects on LDL, these lipid-lowering drugs have additionally been proven to significantly impact vulnerable plaques by inducing plaque regression, de-lipidification, and increasing the thickness of the fibrous cap[45,114-118].

Clinical trials incorporating serial IVUS imaging have shown that potent statin therapy is associated with disease stabilization and plaque regression, typically from 1% to 1.2%, with the degree of effect proportional to the extent of the LDL-C reduction achieved. Additional studies employing IVUS imaging have revealed an added plaque regression by the use of combination therapy with ezetimibe[119].

In our study, none of our patients were using potent LDL-lowering PCSK9 inhibitors. These medications have demonstrated the capacity not only to reduce LDL-c cholesterol by 60%, but also to reduce plaque volume significantly when combined with statin therapy[116,120].

The increased interest in the potential role of plaque phenotype as a fundamental factor in the progression of atherosclerotic disease into clinical manifestations has prompted extensive efforts to analyze how lipid-lowering therapy affects plaque morphology beyond assessing plaque volume alone. As lipid-rich plaques correlate positively with future ischemic cardiovascular events, several pharmacological studies have demonstrated the clinical importance of reducing lipid content in atheromatous plaques[45,120] while no exercise studies have addressed this issue before the CENIT trial.

5.3 HIIT versus pharmacological lipid-lowering – effects on plaque morphology

Among all the phenotypical characteristics of the vulnerable plaque, the lipid core has been identified as having the highest thrombogenic activity[13]. Recent data obtained through advanced imaging techniques suggest that statins may have a more pronounced effect on regulating lipid content within plaques than altering plaque volume[45]. In 2013, The Reduction in Yellow Plaque by Aggressive Lipid-Lowering Therapy (Yellow study)[45] investigated the effect of seven weeks of intensive lipid-lowering therapy (rosuvastatin 40 mg daily) vs. standard-of-care lipid-lowering therapy on plaque composition using NIRS-IVUS in patients with stable angina. Upon follow-up, the intensive statin therapy group achieved a more substantial de-lipidification compared to the standard therapy group. These results suggest that lipid-core content may regress rapidly within a short period of time with intensive statin therapy.

In a recently published small observation study, an enhanced plaque stabilization effect was demonstrated by adding PCSK9i to standard statin treatment in patients with CAD[121]. The results showed a more significant maxLCBI$_{4mm}$ reduction with modern lipid-lowering medication, including PCSK9i, compared to statin monotherapy. Additionally, a significant linear correlation was found between the percent changes in LDL-C and maxLCBI$_{4mm}$. In the recent PACMAN-AMI trial[120], patients receiving combination therapy of alirocumab and statins demonstrated a more substantial reduction in plaque lipid content than those receiving potent statin therapy alone. Effects obtained in lipid-lowering studies on plaque composition are comparable to what we found in Paper I were a de-lipidification was shown in both groups, with a statistically significant reduction only observed in the control group. Of notice, these patients were all on stable lipid-lowering treatment before study start. Accordingly, it could be anticipated maxLCBI$_{4mm}$ was already reduced at the time of inclusion.

Although we did not prove a significant between-group effect on $maxLCBI_{4mm}$ after HIIT compared to usual care, we did observe a moderate positive correlation between increased fitness and de-lipidification even though the LDL-C remained unchanged. The correlation was more pronounced in those improving VO_{2peak} of more than 3.5 $mL \cdot kg^{-1} \cdot min^{-1}$ (1MET). Although this was a secondary post hoc analysis and as such, only hypothesis-generating, it indicates that exercise-induced enhanced fitness may lead to a regression of both coronary plaque volume and lipid content.

5.4 HIIT versus pharmacological lipid-lowering – effects on plaque geometry

The 1.2% coronary plaque regression demonstrated in Paper II appears small at first glance. However, it is similar to the plaque regression accomplished by modern lipid-lowering therapy[114-116] and exceeds the volume reported to impact cardiovascular outcomes[122]. GLobal Assessment of Plaque ReGression with a PCSK9 AntibOdy as Measured by IntraVascular Ultrasound (the GLAGOV-trial)[116] was the first study designed to evaluate the effects of evolocumab on plaque burden in patients with CAD. After 78 weeks of treatment, mean LDL-C levels were significantly lower in the PCSK9 inhibitor group, with a significant greater reduction in PAV and TAV compared to statin monotherapy. In the GLAGOV trial, combination therapy (statin plus evolocumab) resulted in a one percent point greater reduction in PAV vs. placebo (statin monotherapy), similar to our results following HIIT. In the PACMAN-AMI Randomized Clinical Trial[120], the effect of adding PCSK9i (alirocumab) to high-intensity statin therapy (20 mg rosuvastatin) on coronary atherosclerosis in patients with acute myocardial infarction was assessed. The results showed significantly greater PAV regression during 52 weeks of therapy in patients treated with the combination therapy compared with statin monotherapy. The PAV regression in the active treatment group in the PACMAN trial was more substantial compared to previous IVUS trials of statins and the GLAGOV trial. Accordingly, the plaque volume regression observed after HIIT in our study is slightly larger than that observed with statin interventions but less than the demonstrated PAV reduction when adding the PCSK9i alirocumab in the PACMAN trial. It should be emphasized that both the CENIT- and the GLAGOV trials included patients with stable CAD already receiving background statin therapy at the time of the randomization. In contrast, the patients in the PACMAN trial were admitted to the hospital with acute myocardial infarction, and 88% of 300 patients were statin-naive with higher baseline levels of LDL-C.

In summary, our observed effect of exercise as plaque regression-generating intervention is fairly similar to the effects of PCSK9i alone[123]. It is worth noting that the duration of our exercise intervention was half the length of the PACMAN's follow-up. It may be speculated whether a more extended intervention period in the CENIT trial would have resulted in more pronounced plaque regression.

5.5 Individual variations in plaque regression and stabilization

As emphasized in this thesis, the extent of plaque alterations varies among patients. From exercise studies, it is well established that cardiac remodeling depends on individual phenotypical factors, extending beyond exercise frequency, intensity, and duration[84]. Vascular effects may mirror this variability in responses, suggesting that the cumulative exercise burden, quantifiable as enhanced fitness, likely influences the impact on coronary plaques. This hypothesis was elucidated in a sub-analyzes in Paper I, underscoring the impact of enhanced fitness on plaque stabilization as we observed a moderate positive correlation between increased fitness and de-lipidification, even without significant changes in LDL-C and Apo-B. This correlation was more pronounced in individuals showing an improvement in VO_{2peak} of more than 1MET. Although only hypothesis generating, taken together with the results from Paper II, our data adds a potential link between exercise-induced enhanced fitness and the regression of both coronary plaque volume and lipid content.

The phenotypical characteristics of the plaque, encompassing baseline plaque burden and lipid content, are pivotal factors likely to influence the observed variations. Mounting evidence supports that plaque composition influences not only its stability[24] but also the potential for regression[121]. Since the different components, such as lipid content, fibrous tissue, calcium deposits, and inflammatory cells, may respond differently to treatment, a coronary plaque carries varying degrees of regression potential. In general, an unstable plaque phenotype comprising a high-lipid burden tends to be more susceptible to regression than those with a higher fibrous or calcified component. Therefore, patients with ACS have been described to harbour a more modifiable disease substrate which may benefit more from exercise than non-ACS patients. The extent of coronary plaque regression induced by exercise may, in other word, be linked to the coronary plaque phenotype.

Previous pharmacological studies have indicated that statin-induced coronary plaque regression tends to be more pronounced in patients with ACS, with a median percentage change in Total Atheroma Volume (TAV) ranging from -13.1% to -18.1%. In contrast, patients with stable angina pectoris typically exhibit less pronounced changes in TAV, ranging from -0.4% to -6.8%. This hypothesis was further confirmed by the PRECISE-IVUS[119] study, supporting the concept that more substantial plaque regression is notably associated with lower achieved LDL-C levels, especially in the ACS cohort. This suggests a potential association between more potent plaque regression and the acute, unstable presentation of vulnerable patients. Combining exercise training and lipid-lowering treatment may be a particularly effective treatment option for vulnerable plaques. As the patients in our cohort presented with stable coronary disease, one can only speculate that our result would have been even more pronounced in ACS patients.

All patients were on stable lipid-lowering therapy for at least six weeks before inclusion. By then, the effect of statin treatment on LDL-C and Apo-B-containing particles is considered stabilized. As

previously mentioned, a positive correlation between baseline PAV and plaque regression has been demonstrated[123]. As statins reduce PAV by approximately 1-1.2%, the favorable HIIT effects on plaque regression could therefore have been even more substantial if the patients were statin natives as in most of the pharmacological NIRS-IVUS studies.

5.6 Cardiac risk factors and coronary plaque alterations

The presence of specific atherogenic risk factors, including higher baseline HbA1c, higher baseline systolic BP, and lower increase in apolipoprotein A-1, are linked to plaque progression in PCSK9i-IVUS studies[124]. This aligns with previous studies, such as the Treating to New Targets (TNT) study[125], which emphasized the multifactorial nature of atherosclerosis and cardiovascular risk, demonstrating that the determinants of residual risk in statin-treated secondary prevention patients included lipid-related and non-lipid factors such as baseline apolipoproteins, increased body mass index, smoking, hypertension, and diabetes mellitus. Together, these results indicate that despite intensive LDL-C lowering treatment, additional atherogenic risk factors contribute to a greater propensity for plaque progression. These risk factors were equally distributed within the CENIT cohort at baseline. However, the direct impact of potential risk factor alteration during the study period was not further analyzed. In both Papers 1 and 2, we demonstrated improved fitness, reduced waist circumference, and BMI. HDL-C and Apo-A1 concentrations were improved within both groups. These beneficial improvements are associated with several positive cardiovascular effects. Specifically, these changes are linked to reverse cholesterol transport, LDL anti-oxidation, endothelial protection, antiplatelet activity, and anticoagulation[126]. On the other hand, there were no significant changes in HbA1c, LDL-C, and Apo-B. Blood pressure measurements were not included in the analyses.

On the contrary, as highlighted in a scientific statement from AHA, extensive exercise volumes and vigorous intensities are associated with potential cardiac maladaptations, including coronary artery calcification (CAC), myocardial fibrosis, exercise-induced troponin release, and atrial fibrillation[127]. Emerging evidence has demonstrated accelerated coronary artery atherosclerosis among amateur athletes compared with less active healthy controls. In 2008, Möhlenkamp et al.[128] revealed a higher prevalence of coronary artery calcium scores (≥100 Agatston units) in marathon runners compared with matched controls. Aengevaeren et al.[129] supported these findings by demonstrating an increased coronary artery calcium prevalence across progressive tertials of PA volumes in amateur athletes. Of notice, the most active athletes expressed a lower prevalence of unstable mixed plaques. These findings were supported by Merghani et al.[130], suggesting in the MARC-2 study a possible complex relationship where vigorous intensity exercise lowered CAC progression, whereas very vigorous intensity exercise was associated with calcific plaque progression. However, it is worth noting that this study has been criticized for methodological concerns regarding age, risk factors, and statin use.

In the Master@Heart study by De Bosscher et al.[131], the distribution of plaque morphology was similar between the endurance athletes and the controls, with calcific plaque being the most prevalent, followed by mixed and non-calcific types. Furthermore, the study demonstrated that athletes who had engaged in lifelong physical activity were more prone to exhibit significant luminal stenosis (≥50%), particularly in proximal regions, compared to those who started their athletic activities later in life, suggesting that there may be a dose-response relationship between endurance training and the development of coronary artery stenosis. Noteworthy, these studies were limited by the cross-sectional design and thereby unable to determine how vigorous exercise influences the atherosclerotic process.

The mechanisms contributing to coronary artery calcification due to exercise remain incompletely understood. Postulated causes include endothelial repair from repetitive bending and flexing of coronary arteries, inflammation secondary to shear stress, and protracted increases in cardiac afterload. Various factors, including sporting discipline, exercise frequency, intensity and time, blood pressure response, life events, dietary influences, and the potential recall bias associated with retrospective exercise questionnaires, need further assessment as potential confounders[132].

Up to this point, the most compelling evidence suggests that exercise offers overwhelmingly positive effects that extend beyond the cardiovascular system. While current studies have identified a higher prevalence of atherosclerotic plaques in athletes, which correlated with their lifelong exercise levels, it is essential to note that athletes exhibit a superior life expectancy compared to the general population. This phenomenon may be partially attributed to the advantageous coronary adaptations induced by exercise and the less vulnerable plaque morphology. The question of whether extreme levels of exercise have detrimental effects remains open for further investigation. As underscored in a recent review by Fyyaz and Papadakis[132]: "*It is of no surprise that athletes are not immune to the development of CAD, which remains a multifactorial, polygenic disease."*

5.7 Local hemodynamic forces and coronary plaque alterations

When comparing alterations in plaque composition in Paper I, variations in $maxLCBI_{4mm}$ were observed to differ among different coronary artery segments within the same patient. Notably, one-third of the patients exhibited plaques where $maxLCBI_{4mm}$ changed in both directions. However, the baseline $maxLCBI_{4mm}$ was low, and the increase during follow-up was modest. Nonetheless, this non-published data suggests a significant influence of local circulatory factors on plaque alterations. Furthermore, plaque geometry has been shown to undergo similar variations. Madssen et al.[98] demonstrated that plaque vulnerability can change in either direction when comparing distinct lesions within the same coronary artery. Taken together, these observations suggest that local circulatory factors play a crucial role in plaque transformation. This adds to the complexity of assessing plaque biology following exercise, as the potential exercise effects may diverge different coronary segments.

There is increasing evidence that local hemodynamic forces in the coronary tree are pivotal for the dynamic biology of atheroma progression and regression, and it is conceivable that endothelial shear stress may also be involved in the atheroma stabilization and remodeling[89,133].

Shear stress is the frictional force exerted by flowing blood on the endothelial lining of the arteries, varying within different segments of the coronary arteries. A smooth and regular flow pattern characterizes laminar shear stress, whereas disturbed shear stress occurs in turbulent or disturbed flow regions. Laminar shear stress is generally considered to have a protective effect on the arterial wall[89,133,134]. It stimulates nitric oxide production and promotes vasodilation, inhibits platelet adhesion, and reduces inflammation. Certain areas, such as arterial bifurcations, curved segments, and regions downstream from stenosis, are more prone to disturbed flow and altered shear stress[133]. Disturbed shear stress/turbulence can promote endothelial dysfunction, inflammation, and the adhesion of inflammatory cells and lipoproteins to the arterial walls. Turbulent flow transforms the endothelial cells' cellular alignment, expands their permeability to large molecules, and promotes the uptake of the Apo-B-containing LDL, Lp(a), and TG-rich remnant lipoproteins. These regions may exhibit higher susceptibility to plaque formation and progression. Moreover, a low local endothelial shear stress has been associated with atherosclerotic plaque progression independently from their composition[133].
In the Prediction of Progression of Coronary Artery Disease and Clinical Outcome Using Vascular Profiling of Shear Stress and Wall Morphology (PREDICTION) study, low endothelial shear stress (ESS) predicted a progression in PAV. These findings were supported by Eshtehardi et al., demonstrating that coronary segments with PAV $\geq$ 40 % and low ESS particularly increased plaque area to a greater extent than those without low ESS.

The boosted blood flow during HIIT can increase laminar wall shear stress on the coronary endothelium, enabling enhanced responsive vasodilation and alterations in coronary blood flow distribution. These adaptations following bouts of exercise carry implications for shear stress dynamics. The exact mechanisms are intricate and poorly understood.

Intracoronary shear stress measurement is a research method requiring complex and advanced imaging techniques and computational models not routinely performed in clinical practice. Our study did not utilize the technique due to an already comprehensive imaging protocol. The interpretation and clinical significance of shear stress measurements in intracoronary vessels are still evolving and are well-suited to gain insights into the relationship between shear stress and CAD progression. Our data support but do not prove the hypothesis that HIIT may induce plaque alterations through increased coronary ESS from repeated bouts of increased coronary blood flow. Further research is needed to establish standardized protocols and, ideally, utilize non-invasive measurement techniques to expand the research field in exercise research.

In recent times, the prognosis of patients with established coronary atherosclerotic disease has significantly improved due to lifestyle changes, improved pharmacological therapies, and more extensive coronary revascularization. The use of intravascular imaging technologies has enabled the investigation of coronary vessels and have enriched our understanding of the underlying atherosclerotic pathophysiology. Serial imaging trials have demonstrated that more aggressive pharmacological treatments can lead to plaque regression and stabilization. Exercise intervention studies have emerged and shed light on numerous effects of exercise on the heart, but few or none have shown an effect on plaque regression. Our trial demonstrated the direct impact of exercise on diseased coronary vessels as HIIT was found to halt and even counteract the progression of atherosclerotic coronary disease, promoting plaque regression and potentially even enhancing stabilization. Moreover, HIIT proved to enhance cardiovascular health, resulting in a notable increase in VO_{2peak} and reductions in BMI. Such findings contribute to our understanding of the positive influence of exercise on coronary artery disease. Together, these outcomes reinforce the scientific basis for including physical exercise training as a crucial component of CR programs and rationalize the support of adopting exercise training as a treatment regimen for patients with atherosclerotic coronary disease.

Further research is needed to establish a more evident correlation between HIIT and the extent of plaque regression and stabilization. In particular, the relationship between HIIT, atheroma burden, lipid content, and cardiovascular outcomes still requires elucidation. Understanding the biological changes in coronary plaque phenotype could unveil the mechanistic aspect and bridge the gap between exercise training with enhanced fitness and the observed reduction in cardiovascular outcomes in large-scale clinical trials. Future studies will be essential in comprehending the practical implications of these findings and developing new non-invasive techniques for detecting specific features of coronary plaques. Whether HIIT, following PCI for CAD treatment, provides a prognostic advantage remains to be determined.

5.8 Biomarkers for lipid content- is the answer lipoprotein subfractions?

While conventional risk prediction models rely on major cardiovascular risk factors identified within affected populations, there remains a lack of precise biomarkers specific to CAD. Understanding the interplay between biomarkers and vulnerable plaques helps in risk assessment and guiding therapeutic interventions. While advanced intracoronary imaging techniques like NIRS-IVUS can assess plaque composition, its widespread application in contemporary clinical practice has not been established due to its invasive nature. Consequently, a clinical imperative exists to identify non-invasive biomarkers that effectively reflect lipid content within coronary plaques.

In Paper III, we found no significant associations between lipoprotein subfractions, as evaluated by nuclear magnetic resonance spectroscopy, and coronary plaque lipid content ($maxLCBI_{4mm}$) by NIRS. In an exploratory analysis, serum levels of Lp(a) and free cholesterol within the smallest HDL subfractions (HDL-4) emerged as the lipoproteins displaying the most substantial potential as predictors for coronary plaque lipid content. While the evidence is preliminary, our study implies that assessing lipoprotein subfractions might offer supplementary insights into coronary plaque composition beyond traditional lipid measurements. However, upon incorporating established cardiovascular risk factors into the regression model, none of the lipoproteins qualified as potential predictors of $maxLCBI_{4mm}$. In other words, it does not surpass the predictive capacity of established risk factors in patients on lipid-lowering therapy. We did not detect any associations between traditional lipid measurements and coronary lipid content.

In the past, despite dyslipidemia being acknowledged as a pivotal risk factor for CAD, only a few studies have examined the relationship between lipoprotein subfractions and the features of vulnerable coronary plaques assessed by advanced intracoronary imaging. A study from Honda et al., involving patients with acute or stable CAD, demonstrated that change in HDL-C, but not other lipids, was associated with changes in coronary plaque lipid burden assessed by NIRS. Over a median follow-up of 13 months, there was a significant decrease in max $LCBI_{4mm}$. On univariable analysis, the percent change in HDL-C was negatively associated with the change in max $LCBI_{4mm}$[135]. Notably, none of the patients experiencing an increase in HDL-C demonstrated progression of lipid core plaque. Conversely, almost one-third of patients who did not experience an increase in HDL-C exhibited plaque lipid progression despite receiving statin therapy. In contrast, no significant associations were observed between other lipid parameters, such as LDL-C levels, and changes in lipid core. A potential explanation for these findings was that, as for the CENIT trial, most study patients (78%) were already on statins, and the median baseline LDL-C levels were relatively low. These findings underscore the potential significance of HDL in influencing the serial evolution of plaque composition and are in line with previous autopsy studies showing that low HDL-C levels were associated with an increased number of vulnerable plaques harboring a large lipid pool[136].

Another NIRS-IVUS study reproduced a similar finding, identifying a negative correlation between HDL-C and maxLCBI$_{4mm}$ in TCFA lesions[137]. A subsequent analysis, based on the Lipid Rich Plaque Study[24], encompassing 984 patients, explored potential correlations between maxLCBI$_{4mm}$, LDL-C, and HDL-C. Torguson et al. could not show any relationship between NIRS-IVUS-measured maxLCBI$_{4mm}$ and serum-measured LDL-C in statin-treated patients. At the same time, a weak correlation did exist for statin-naïve patients, but they could not show any relationship between NIRS-IVUS-measured maxLCBI$_{4mm}$ and serum-measured HDL-C[138].

These findings correspond with the observations in Paper III, indicating that none of the standard lipid measurements showed an association with the lipid content within coronary atheromatous plaques. This lack of association could be attributed to the influence of lipid-lowering therapy.

Mounting evidence emphasizes that HDL function is more critical in atherogenesis and atheroprotection than absolute HDL-C levels. Consequently, the recent direction in HDL research has shifted towards strategies to enhance HDL functionality rather than solely boosting HDL-C levels. Further comprehensive studies are warranted to elucidate the impact of HDL functionality on changes in plaque composition in vivo.

In our study, Lp(a) and free cholesterol in HDL4 emerged as the most prominently linked lipoprotein subfractions to coronary lipid content. This observation implies that statins might not significantly influence these particular subfractions. Furthermore, they could potentially offer supplementary insights into coronary plaque composition beyond what is gleaned from traditional lipid measurements.

The study conducted by Nakamura et al.[139] recently revealed an association between Lp(a) and maxLCBI$_{4mm}$ in patients diagnosed with CAD and diabetes. Interestingly, this association was not observed in patients with CAD but without diabetes. Lp(a) is recognized as a significant contributor to plaque vulnerability, partially attributed to its binding to oxidative phospholipids, which possess pro-inflammatory properties and harbor Apo-B glycation. These mechanisms are known to be heightened in diabetic patients.

Our study represents a pioneering effort in exploring the association between a comprehensive range of lipoprotein subfractions and the lipid content within coronary atheromatous plaques, as measured by maxLCBI$_{4mm}$ by NIRS. By delving into a wide array of lipoprotein subfractions, this study aimed to provide novel insights into the relationship between circulating lipoproteins and the lipid composition of coronary plaques. The utilization of NIRS for assessing maxLCBI$_{4mm}$ adds further significance to this research, as it allows for direct evaluation of plaque lipid burden in a precise and efficient manner. Further extensive studies with larger study samples are imperative to evaluate the potential of circulating lipoprotein subfractions as consequential biomarkers, both for lipid content in coronary atheromatous plaques and as markers for CAD.

5.9 General methodological considerations

RCTs using serial intracoronary imaging are challenging to conduct, and only a few exercise studies use this acquisition method for outcome measurements. When planning the CENIT trial, there were no published data on the effect of physical exercise on $maxLCBI_{4mm}$. At that time, RCTs using NIRS imaging were relatively uncommon, and information regarding chemograms likewise. For these reasons, power calculations were based on earlier IVUS studies from our research group and balanced against what was practically feasible.

In Paper I, the study arms became unbalanced after excluding patients with low intracoronary lipid content at baseline. The rejection of these 11 patients amplified a power problem concerning the low number of patients included in the first place. This could have been avoided by only including patients with high lipid content in residual plaques.

It is well known that confounding and selection biases challenge the validity of exercise studies as participants generally tend to be younger, predominantly male, with fewer comorbidities, and fitter than the general population. It is worth noting that the patients in our studies are comparable to participants in CR exercise trials. Selection biases are modulated during the randomization process, and to make the treatment groups well-balanced, we stratified the patients by age and gender.

Our study randomized the patients after baseline data collection was complete. With block-randomization, we ensured that each patient had the same probability of being placed in the HIIT as in the control group. To avoid confounding, all patients had to be on stable lipid-lowering treatment for at least six weeks before entering the study. Due to variations in risk scores and disease burden within the study population, the duration of lipid-lowering therapy was different. However, the randomization process neutralized this disparity. As we were concerned about how variations in anti-inflammatory medicine and other unknown factors could affect and change the outcome variables, autoimmune diseases were set as an exclusion criterion.

Blinding patients, investigators, and caregivers for the allocation result is beneficial to minimize the risk of performance bias; this was impossible in the CENIT study due to the design. The possibility of detection bias was diminished by the use of an independent core laboratory (KCRI, Krakow, Poland), blinded for patient characteristics and randomization. There were few dropouts in the CENIT population.

External validity describes the extent to which the results of an RCT can be generalized into clinical practice, which can be jeopardized if the inclusion and exclusion criteria settings are too strict. Due to practical reasons, we lack an overview of the number of patients who were found eligible for

participation, those who did not meet the inclusion criteria, those who did not wish to participate, and possibly other reasons for not joining the study. The main reason why patients were not found eligible for inclusion was the lack of statin use. Practical conditions and challenges surrounding the intervention were the most common causes of patients' refraining from participating.

In the CENIT study, women were significantly underrepresented, with only 8% participation. Male subjects have dominated exercise science for many years, and women do not participate in CR to the same degree as men. Reasons for this are multifactorial, but in the CENIT population, this limitation was mainly because fewer women were admitted to the hospital for stable angina. This low attendance rate by women, few older adults, and the probability of selection bias affects the external validity and must be kept in mind when evaluating the generalizability of the results.

5.10 Strengths and limitations

There are some limitations that needs to be addressed. RCT design ranks at the top of the evidence pyramid, categorizing evidence by robustness, where only meta-analyses and systematic reviews have a higher impact[140]. However, using this research method in exercise research is not without concerns. A major challenge in studies comparing two different exercise protocols is that exercise levels between study groups do not differ as much as desired.

This is, to some degree, the fact also in the CENIT trials. We observed enhanced cardiopulmonary fitness in both arms, reflecting the well-known methodological difficulties with group contamination in randomized controlled exercise trials. The tendency for individuals who enroll in these studies to increase their exercise level weakens the scientific ability to isolate physical activity as a variable. On the other hand, given its benefits, it would be unethical not to promote physical activity for the patients. From a methodological point of view, using a passive control group, unaware of being observed, would be ideal. For practical and ethical reasons, that would not be feasible.

To avoid reinforcing the Hawthorn effect, only the intervention group received the heart rate monitoring tool, primarily used for intensity tracking and as a motivator to maintain increased physical activity. Another limitation is that we did not collect data from the wearable devices. However, we admit that as the quality of the devices increases, this will be an exciting data tool for future exercise research. There was no contact with the control group during the study period. Accordingly, we have no information regarding their exercise habits.

Another obvious limitation, besides the small sample size, is the exclusion of patients with LCBI < 100 in Paper I, which led to an imbalance between the groups. We did not face this problem in Paper II, as the atheroma volume analysis could be performed in all patients with valid recordings.

Last but not least, as already mentioned, there was a lack of female participants. Although the sample size is relatively small, our study has more participants and a more extended intervention period than most exercise RCTs. A more prolonged intervention period would have been preferable, as we know from lipid-lowering therapy trials that the beneficial effect on coronary plaque burden and composition increases with time. Therefore, our results may be underestimated due to only six months of follow-up, and by that, limited to short-term exercise rather than chronic habitual exercise.

A strength of this study is that it was conducted as an integrated part of a clinical patient pathway. Additionally, patients were on stable lipid-lowering therapy for at least six weeks before inclusion, with no change in this medication throughout the study. Furthermore, blinded IVUS data were analyzed at an independent core laboratory, and validated exercise protocols in line with contemporary guidelines were used. Of note, the training intervention was carried out by physiotherapists and not in a research laboratory. Thus, the study design corresponds well with contemporary exercise-based CR and strengthens the transferability. Although the studies have some limitations, we consider the collected data reliable and generalizable. We find both internal and external validity robust, and the results relevant and applicable to other patients with stable coronary disease.

Paper III has some additional limitations, including a small sample size and the extensive analysis of numerous subfractions. The study specifically focused on the single most diseased lesions, overlooking the disease status of the entire coronary tree.
Lp(a) levels are associated with an increased risk for CHD events in patients with CAD[141]. Multiple prospective and epidemiological studies have convincingly demonstrated a causal and linear relationship between high Lp(a) concentrations and various CVD outcomes. Key features of these studies are the graded positive association between Lp(a) and events[142]. In Paper III, we dichotomized the variable into normal versus abnormal levels. Accordingly, we have most likely weakened the association by not using Lp(a) as a continuous variable.
Despite these limitations, a strength of the study lies in the utilization of advanced coronary imaging technology, coupled with data interpretation conducted by an independent core facility.

6. CLINICAL IMPLICATIONS

A solution to the controversy regarding coronary artery disease and exercise training, which centers on the paradox of exercise, has been sought for decades. Intravascular plaque imaging and cardiac exercise research are challenging domains. Papers I and II provides essential contributions to the scientific background of the beneficial effects of exercise training in patients with stable CAD. We found that HIIT induced favorable plaque regression and improved cardiovascular health by increasing cardiopulmonary fitness while reducing waist circumference and BMI. Additionally, although the evidence is secondary and correlative, our research advocates that increased CRF may decrease lipid content in coronary plaques, making the plaques less vulnerable.

These studies bring us closer to an understanding of how exercise reduces the risk of cardiovascular outcomes. The data endorses current exercise recommendations, as exercise, particularly HIIT, not only seems to induce improvements in traditional cardiovascular risk factors but may also confer some benefits through favorable changes in plaque characteristics.

7. FUTURE PERSPECTIVES

The evidence for exercise training to reduce coronary atheroma burden and favorably alter plaque composition is strengthened with the CENIT trial. However, the underlying mechanisms for plaque stabilization and regression are still unknown. There is a paucity of natural history studies of atherosclerosis in humans, and the precise role of WSS in plaque evolution still needs to be determined.

A hope for the future is that modern CT-technology may open for new methods to assess coronary atheromatous plaques in larger populations over time to further analyze potential correlations between plaque characteristics and clinical endpoints.

In the initial phase of planning our study, we sought to use OCT imaging to assess the effect of exercise on vulnerable plaques. However, to perform three-vessel NIRS-IVUS and OCT would result in a comprehensive invasive protocol and ethical considerations made us omit that plan. However, in a study design like ours, there is no doubt that OCT could yield additional and important knowledge of plaque behavior. Accordingly, a catheter, including all three modalities, would be ideal for future studies.

However, invasive investigations for scientific purposes have obvious limitations. The manifestation of large lipid pools, thin fibrous caps, and marked inflammatory cell infiltrations are critical features of coronary plaques susceptible to rupture and generate coronary events. There is a need to discover biomarkers that reflect these coronary plaque phenotypes. Before lipoprotein subfractions can be used in the clinic, a standardized method for lipidomic analyses is required.

Overall, ongoing research in imaging modalities and their correlation with plaque lipid content will continue to advance our understanding of atherosclerosis and improve patient care by enhancing risk stratification and guiding optimal preventive treatments.

8. CONCLUSIONS

This thesis expands prior knowledge and contributes to understanding the relationship between exercise training and atherosclerotic coronary artery disease.

- In the CENIT trial, we found no significant difference in the change of coronary plaque composition between 6 months of supervised HIIT versus standard care for patients with stable coronary artery disease.

- By NIRS, the lipid content measured as $maxLCBI_{4mm}$ showcased a numerical decrease in both groups but slightly more pronounced and significant only in the control group.

- A moderate negative correlation between VO_{2peak} and a change in lipid content was demonstrated, generating the hypothesis that exercise with a subsequent increase in fitness may induce de-lipidification of coronary atheromatous plaques.

- When assessing the effect of HIIT on coronary plaque geometry by IVUS, plaque regression was observed in those undergoing six months of supervised high-intensity interval training compared with patients following contemporary preventive guidelines.

- Our examination of the association between lipoprotein subfractions and coronary lipid content in statin-treated patients with stable CAD revealed that Lp(a) and free cholesterol within the smallest HDL subfractions, HDL-4, displayed the highest promise as potential predictors of coronary lipid content. However, only moderate evidence was demonstrated, and these associations weakened when adjusting for established risk factors.

These results reinforce the scientific background for recommending physical exercise as an essential component of prevention and highlight the importance of including exercise prescriptions in addition to optimal medical intervention in CR programs.

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PAPER I

Journal of the American Heart Association

ORIGINAL RESEARCH

CENIT (Impact of Cardiac Exercise Training on Lipid Content in Coronary Atheromatous Plaques Evaluated by Near-Infrared Spectroscopy): A Randomized Trial

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BACKGROUND: The effect of physical exercise on lipid content of coronary artery plaques is unknown. With near infrared spectroscopy we measured the effect of high intensity interval training (HIIT) on lipid content in coronary plaques in patients with stable coronary artery disease following percutaneous coronary intervention.

METHODS AND RESULTS: In CENIT (Impact of Cardiac Exercise Training on Lipid Content in Coronary Atheromatous Plaques Evaluated by Near-Infrared Spectroscopy) 60 patients were randomized to 6 months supervised HIIT or to a control group. The primary end point was change in lipid content measured as maximum lipid core burden index at 4 mm ($maxLCBI_{4mm}$). A predefined cutoff of $maxLCBI_{4mm}$ >100 was required for inclusion in the analysis. Forty-nine patients (HIIT=20, usual care=29) had $maxLCBI_{4mm}$ >100 at baseline. Change in $maxLCBI_{4mm}$ did not differ between groups (–1.2, 95% CI, −65.8 to 63.4, P=0.97). The estimated reduction in $maxLCBI_{4mm}$ was −47.7 (95% CI, −100.3 to 5.0, P=0.075) and −46.5 (95% CI, −87.5 to −5.4, P=0.027) after HIIT and in controls, respectively. A negative correlation was observed between change in peak oxygen uptake (VO_{2peak}) and change in lipid content (Spearman's correlation −0.44, P=0.009). With an increase in VO_{2peak} above 1 metabolic equivalent task, $maxLCBI_{4mm}$ was on average reduced by 142 (−8 to −262), whereas the change was −3.2 (154 to −255) with increased VO_{2peak} below 1 metabolic equivalent task.

CONCLUSIONS: Six months of HIIT following percutaneous coronary intervention did not reduce lipid content in coronary plaques compared with usual care. A moderate negative correlation between increase in VO_{2peak} and change in lipid content generates the hypothesis that exercise with a subsequent increase in fitness may reduce lipid content in coronary atheromatous plaques.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT02494947.

Key Words: coronary atheromatous plaques ■ lipid core burden index ■ near infrared spectroscopy ■ physical exercise

See Editorial by Guseh et al.

Physical exercise is highly recommended for secondary prevention of coronary artery disease (CAD),[1] and multiple beneficial biological effects are demonstrated with regular physical exercise.[2] Furthermore, several studies have demonstrated strong and positive associations between the level of physical exercise and life expectancy both in healthy subjects and in patients with CAD.[3,4] However, survival benefit from regular physical exercise has not been demonstrated in randomized clinical trials in CAD. There is a paucity of data on the effect of physical exercise on coronary artery atheromatous plaques. Studies on athletes with coronary computed tomography angiography indicate that strenuous physical exercise is associated with increased coronary artery calcium.[5,6] Data regarding the effect of physical

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CLINICAL PERSPECTIVE

What Is New?

- Physical exercise following percutaneous coronary intervention in patients with stable coronary artery disease may reduce lipid content in coronary atheromatous plaques assessed by near infrared spectroscopy with a potential positive correlation between increase in VO_{2peak} and reduction in lipid content.

What Are the Clinical Implications?

- A lipid-reducing effect of physical exercise in coronary atheromatous plaques reinforces the scientific evidence for recommending physical exercise in rehabilitation programs.

Nonstandard Abbreviations and Acronyms

HIIT	high intensity interval training
LCBI	lipid core burden index
NIRS	near infrared spectroscopy
PROSPECT	Providing Regional Observations to Study Predictors of Events in the Coronary Tree

exercise on plaque volume, plaque composition, and plaque vulnerability are modest. With radiofrequency intravascular ultrasound, we previously demonstrated a reduction in necrotic core following a period of exercise training in patients with CAD.[7] Furthermore, we demonstrated that plaque vulnerability following an exercise intervention could change in different directions within the same patient, indicating that local intracoronary factors affect plaque composition. Near infrared spectroscopy (NIRS) can be used to determine lipid content in coronary artery atheromatous plaques.[8] Previous studies have demonstrated that lipid-rich lesions are more vulnerable and together with plaque volume represent increased risk for future cardiac events,[9–13] recently also demonstrated in the PROSPECT 2 (Providing Regional Observations to Study Predictors of Events in the Coronary Tree) trial.[14] With the use of NIRS, pharmacotherapy studies with lipid-lowering drugs have confirmed a favorable reduction in coronary artery lipid content.[15,16] No previous trial has investigated the effect of physical exercise on lipid content in coronary arteries. In this trial we hypothesized that lipid content in coronary atheromatous plaques assessed with NIRS would be reduced after a period of regular high intensity interval training (HIIT) when compared with usual care.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Design and Patient Population

This study was an investigator-initiated, single-center, open, parallel, randomized controlled trial undertaken at the Clinic of Cardiology, St. Olavs University Hospital in Trondheim, Norway. The study protocol was approved by the Regional Ethics Committee of Central Norway (2015/210), registered at clinicaltrials.gov (NCT02494947), and performed according to the Declaration of Helsinki. Written informed consent was obtained from all participants.

Sixty patients with symptomatic stable CAD treated with percutaneous coronary intervention (PCI) were included. The inclusion criteria were use of statins for at least 6 weeks before undergoing PCI and being able to perform the prescribed exercise program. Exclusion criteria were previous coronary artery bypass surgery, known inflammatory disease other than atherosclerosis, planned surgery within the next 6 months, inclusion in another randomized trial or inability to comply with the study protocol due to any somatic disease, physical disability, mental problems, or already performing physical activity at a similar or higher level than the prescribed activity for the intervention group. Guideline-directed medical therapy was administered to all patients following PCI. However, no adjustments were made for lipid-lowering therapy throughout the study. After completion of baseline acquisitions, patients were randomly allocated to a supervised 6 months intervention with HIIT or advised to follow the current recommendations for secondary prevention in CAD with no protocol-based follow-up until the end of the study period. Randomization was performed by a web-based randomization system developed and administered by the Unit of Applied Clinical Research, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway.

Exercise Training

The intervention group joined supervised endurance training performed as walking or running on a treadmill or bicycling following the HIIT-principle 2 times weekly for 6 months. Each session started with a 10-minute warmup at moderate intensity (60% to 70% of peak heart rate), followed by 4×4 minute intervals with an intensity at 85% to 95% of peak heart rate with 3 minutes of active recovery at moderate intensity between intervals, ending with a 5-minute cooldown period.[17] In addition, patients were encouraged to perform

home-based endurance training with bouts of intervals, and any activity mode involving dynamic work with large muscle groups was recommended. To ensure adequate training intensity and increase adherence, the patients in the HIIT group were encouraged to use a wearable device for heart rate and activity tracking (Mio Global PAI, Toronto, Canada). The control group was encouraged to practice regular physical activity of moderate intensity according to contemporary guidelines[1] without receiving any further supervision.

Study Outcomes

The primary outcome was change in lipid content in matched coronary atheromatous plaques from baseline to follow-up measured by NIRS. Secondary outcomes were changes in cardiorespiratory fitness measured as peak oxygen uptake (VO_{2peak}), body mass index, waist circumference, blood lipids and glycosylated hemoglobin A1c.

Invasive Procedures

Coronary angiography and PCI were performed according to standard techniques, and a standard transthoracic echocardiography was performed at baseline following PCI. Drug-eluting stents were used in all cases. After successful treatment of all flow-limiting lesions, 3-vessel intravascular imaging of epicardial vessels was performed when feasible by a 3.2 Fr NIRS catheter using an automated pullback system (TVC-MC8 model system with a 3.2Fr 40 MHz catheter, Infraredx, Burlington, MA). Following administration of intracoronary nitroglycerine, the NIRS catheter was positioned as distally as possible in the coronary artery. The distal starting points of the pullbacks were recorded angiographically to assist in registration of the corresponding segments at follow-up. Untreated lesions were identified, and their plaque morphology was evaluated by NIRS. NIRS spectroscopic data generate a chemogram where data are illustrated by a color-coded distribution from red to yellow. Yellow pixels represent the highest probability of lipid deposition and allows for a calculation of lipid core burden index (LCBI) that ranges from 0 to 1000 equivalent to the percentage of yellow pixels in the segment of interest.[18]

Angiograms and intravascular ultrasound data were analyzed at an independent core laboratory, (KCRI, Krakow, Poland), without any knowledge of patient characteristics or randomization allocation. Intravascular data were analyzed using the commercial software (Pie Medical Imaging Software, CAAS Intravascular). Analyses were performed for every 0.5 mm of the arteries. Regions of interest were defined as a target segment lesion with an atherosclerotic plaque compromising the lumen by at least 30% by cross-sectional area, with the 5 mm segments proximal and distal to the target segment as reference. The corresponding regions of interest at baseline and follow-up were identified using fiduciary points such as side branches and the implanted stent, as well as comparison of angiographic records. LCBI was estimated for the analyzed segments and the change in maximal value of LCBI per 4 mm ($maxLCBI_{4\ mm}$) in matched coronary segments at baseline and follow-up was defined as the primary end point. The plaque with the highest $maxLCBI_{4\ mm}$ at baseline was used for further analysis irrespective of where in the coronary artery tree it was located.

Cardiovascular Risk Factors

Medical history uptake, physical examination, anthropometrics, fasting blood sampling, cardiopulmonary exercise testing, and echocardiography were performed at baseline and follow-up.

Exercise Testing

Two weeks following PCI, and after the study period, an individualized cardiopulmonary exercise testing was performed to determine exercise tolerance, VO_{2peak}, and peak heart rate in order to tailor exercise prescription and evaluate training effects. Data from the 12-lead ECG, blood pressure, and respiratory gases (Jaeger Vyntus CPX, Hoechber, Germany) were recorded continuously. The individualized steady-state test protocol started at a speed and inclination set during the 15 minutes warmup guided by the Borg scale. Thereafter workload was increased either by 1 km/h in speed or by 2% every minute until the participant stopped the test due to exertion or VO_{2max} was achieved. The protocol lasts 8 to 12 minutes. VO_{2max} was defined as a flattening of the oxygen uptake curve despite an increased workload combined with a respiratory exchange ratio above 1.05.

Statistical Analysis

In the planning of this study, we had no data on the effect of physical exercise on $maxLCBI_{4mm}$. In a previous study using radiofrequency intravascular ultrasound (IVUS) of 36 patients undergoing PCI, we demonstrated a reduction in necrotic core both for patients undertaking 12 weeks of HIIT and those exercising at moderate intensity with no in-between group difference.[7] Based on this experience, we designed the present study with a sample size of 60 and prolonged the intervention from 3 to 6 months.

Statistical analyses were performed using the SPSS version 26.0 (IBM Corporation) and Microsoft Office Excel 2016. Baseline clinical characteristics are presented as means with SDs or as frequencies and percentages. Normally distributed continuous variables

were compared using the Student *t*-test and categorical variables were compared by the chi-square test or Fisher's exact test. For the comparison of the primary end point, change in maxLCBI$_{4mm}$ (ΔmaxLCBI$_{4mm}$) between groups, we specified a linear mixed model with time and group as fixed effects variables. Dependence of observations within individuals was handled by including individual-specific random intercepts. Owing to randomization, mean outcomes at baseline were in the models constrained to be equal between the groups.[19] Similar models were used for the secondary end points. To study the relationship between the change in VO_{2peak} (ΔVO_{2peak}) and ΔmaxLCBI$_{4mm}$ from baseline to follow-up, the Spearman's correlation coefficient was calculated. *P* values <0.05 were considered statistically significant and CIs are presented at the 95% level. Normality of residuals was checked by visual inspection of normal QQ-plots.

RESULTS

Study Patients and Lesion Characteristics

From February 2016 to April 2019, 60 patients with stable CAD on statin treatment for at least 6 weeks before undergoing PCI were found eligible and enrolled in the study. In patients with no atheromatous plaque with maxLCBI$_{4mm}$ >100 at baseline, lipid content was considered too low to predict alterations in plaque composition with certainty. Accordingly, 11 patients were excluded. Thus, 49 patients (20 in the HIIT group and 29 in the control group) with a total of 142 imaged coronary arteries at baseline (mean 2.9 per patient) were included and analyzed for the primary end point (Figure 1). During the intervention period, 1 patient in the HIIT group developed severe angina pectoris and underwent coronary artery bypass grafting and another patient in the HIIT group emigrated. Also, 1 patient in the HIIT group had unsuccessful NIRS-IVUS imaging at follow-up. Thus, there were no follow-up NIRS-IVUS data from these 3 patients. One patient in the control group suffered a minor stroke at the end of the follow-up period; otherwise there were no adverse events during the study. There were no complications related to the invasive procedures. Baseline patient characteristics were similar between the 2 groups (Table 1). The patients were middle aged and cardiovascular risk factors were prevalent. All patients were on dual antiplatelet therapy and stable statin treatment.

Primary End Point: Changes in maxLCBI$_{4mm}$

At baseline, the maxLCBI$_{4\ mm}$ was well balanced between the 2 groups (Table 2). The artery with the highest maxLCBI$_{4\ mm}$ was the left anterior descending artery (including the first diagonal branch) in 21 patients, the right coronary artery in 13 patients, the circumflex artery in 14 patients, and the intermediate artery in 1 patient. There was no significant difference in the change of maxLCBI$_{4\ mm}$ from baseline to follow-up between the HIIT and the control group (–1.2; 95% CI, −65.8 to 63.4; *P*=0.97), and a similar mean reduction in maxLCBI$_{4mm}$ was observed in both groups (Table 3). Figure 2 demonstrates an imaged vessel with coronary angiography and NIRS-IVUS. When comparing maxLCBI$_{4mm}$ between different arteries in the same patient, we observed that lipid content in atheromatous plaques changed in both directions in 16 patients at follow-up. No consistent pattern with respect to changes in maxLCBI$_{4mm}$ based on the anatomical origin of the coronary artery was observed.

Changes in maxLCBI$_{4mm}$ Relative to VO_{2peak}

When analyzing patients with increased peak oxygen uptake at follow-up, a moderate negative correlation (Spearman's correlation −0.44, *P*=0.009) between ΔVO_{2peak} and ΔmaxLCBI$_{4mm}$ was found (Figure 3). Among those with an increase in VO_{2peak} above 1 MET (3.5 mL·kg^{-1}·min^{-1}) maxLCBI$_{4mm}$ were on average reduced by 142 (range –8 to –262), whereas the change was –3.2 (range 154 to –255) among those with an increased VO_{2peak} below 1 MET. Among the 17 patients with an increased VO_{2peak} below 1 MET, 9 had increased and 8 decreased LCBI$_{4mm}$ (Figure 3).

Cardiovascular Risk Factors

Cardiovascular risk factors at baseline and follow-up are presented in Table 2. There was a larger increase in VO_{2peak} in the HIIT group compared with the control group (*P*=0.034). VO_{2peak} increased significantly after HIIT (*P*<0.001) but not in the control group (*P*=0.113, Table 3). The reduction in body mass index and waist circumference was also significantly larger after HIIT compared with the control group. In both groups a modest although significant increase was observed for both high-density lipoprotein cholesterol and apolipoprotein A1 during the intervention period. There were no changes in glycosylated hemoglobin A1c levels.

DISCUSSION

The CENIT (Coronary Disease and the Effect of High-Intensity Interval Training) study is the first study to assess potential effects from exercise training on lipid content in coronary artery atheromatous plaques by NIRS. Main findings were (1) we did not observe any effect of HIIT compared with usual care on maxLCBI$_{4mm}$; lipid content was reduced by similar amounts in both groups, with a slightly stronger evidence of a difference

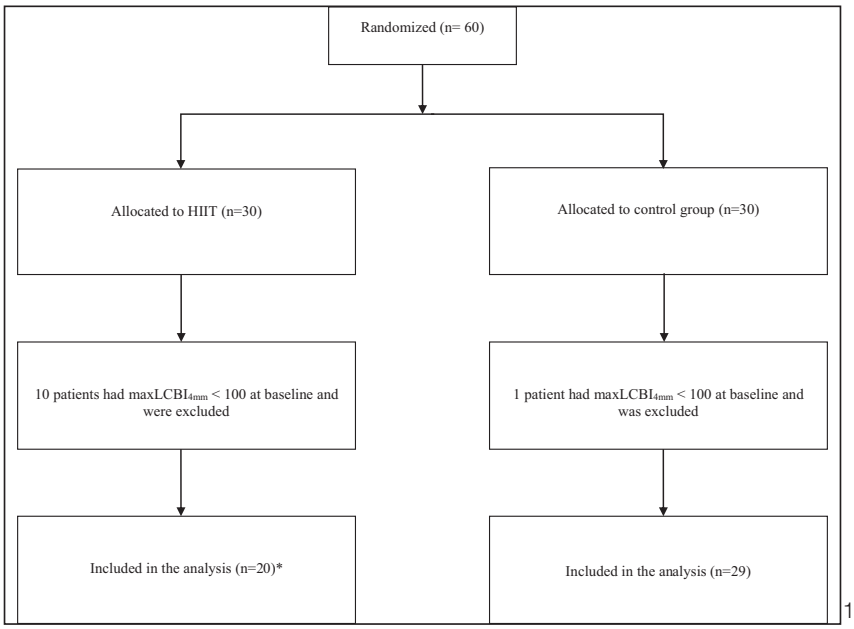

Figure 1. Flow diagram of randomization, allocation and analysis of study data.
Flow chart illustrating enrolment, randomization, allocation, and follow-up throughout the study. *At 6 months follow-up 1 patient had emigrated, 1 patient underwent coronary artery bypass grafting, and in 1 patient the near-infrared spectroscopy chemogram was not interpretable. HIIT indicates high intensity interval training; and maxLCBI$_{4mm}$, maximum lipid core burden index within any 4 mm segment across the entire lesion.

in the control group; (2) we observed a moderate positive correlation between increase in VO_{2peak} and reduction in lipid content; and (3) when analyzing patients with increased VO_{2peak} at follow-up, a larger reduction in maxLCBI$_{4mm}$ was observed in patients with an increase in VO_{2peak} above 1 MET compared with patients with an increased VO_{2peak} below 1 MET.

Although physical exercise is considered a cornerstone in cardiac rehabilitation, only a few randomized trials using intracoronary imaging for assessing the effects of physical exercise on atherosclerosis progression have been conducted, typically with a limited number of patients included.[20–22] This reflects the complexity, resource demands and patient safety aspects of such studies. The rationale for our study has gained increased importance as the recently published PROSPECT 2 trial demonstrated maxLCBI4$_{mm}$ in coronary plaques to be a risk factor for subsequent acute coronary events.[14] In an earlier study from our group,[7] exercise-induced changes on coronary plaque geometry and composition following 3 months of exercise were evaluated by greyscale- and radiofrequency IVUS. In that study, aerobic exercise for 12 weeks following PCI induced a reduction in necrotic core, a marker of plaque vulnerability. Based upon this study, we expected that 6 months of HIIT would reduce lipid content more than usual care in the present study applying a novel technology like NIRS. However, change in maxLCBI$_{4mm}$ did not differ between groups in our study. A challenge in trials comparing exercise protocols with different intensity is that the control group tends to increase their physical activity level, thus masking the effect of the intervention, as observed in a previous exercise trial.[23] In our study 15 out of 29 controls with a maxLCBI$_{4mm}$ >100 at baseline increased their VO_{2peak} more than 1 mL·kg^{-1}·min^{-1} in the study period, an increase that has been considered clinically relevant in previous studies.[24] We found an increase in VO_{2peak} of more than 3.5 mL·kg^{-1}·min^{-1} (1 MET) increased the probability of reduced lipid content in coronary plaques (Figure 3). However, this was a secondary post hoc analysis and as such only hypothesis generating.

Table 1. Baseline Characteristics

	Exercise group HIIT (n=20)	Control group (n=29)
Characteristics		
Age, y	57.6±6.2	58.4±7.4
No. of men/women	19/1	26/3
Body mass index, kg/m²	29.1±4.4	29.2±3.6
Medical history, n (%)		
Hypertension, medically treated	9 (45)	15 (52)
Hyperlipidemia	6 (30)	15 (52)
Diabetes	1 (5)	5 (17)
Smoking currently	1 (5)	4 (14)
Smoked previously	11 (55)	15 (52)
Heredity for premature cardiovascular disease	18 (90)	22 (76)
Prior history of coronary artery disease	11 (55)	15 (52)
Congestive heart failure	1 (5)	2 (7)
Left ventricular ejection fraction, %	53±3	52±3
Medication at baseline, n (%)		
Dual antiplatelet therapy	20 (100)	29 (100)
Statins	20 (100)	29 (100)
Combined therapy with ezetimib	1 (5)	3 (10.3)
β-blockers	8 (40)	10 (34.5)
Angiotensin-converting enzyme inhibitors/ angiotensin II receptor antagonists	9 (45)	14 (48.3)

Values are presented as mean±SDs for continuous variables and as frequencies (%) for categorical variables. HIIT indicates high intensity interval training.

There are no previous exercise trials to compare these data with. However, it has been documented that lipid content measured by NIRS decreases during intensive pharmacological cholesterol lowering therapy. Changes in plaque lipid content after short-term intensive statin therapy versus standard statin therapy were described in the YELLOW (Reduction in Yellow Plaque by Aggressive Lipid-Lowering Therapy) trial.[15] Comparing with the YELLOW trial, patients in our study increasing their VO_{2peak} with more than 1 MET

Table 2. Outcomes

	Exercise group HIIT (n=20)		Control group (n=29)	
Outcome	Baseline	Follow-up	Baseline	Follow-up
Maximum lipid core burden index within any 4 mm segment	357±136	306±150	336±157	292±186
Exercise testing				
VO_{2peak}, mL·kg^{-1}·min^{-1}	32.3±5.9	36.2±7.0	29.1±6.2	30.6±7.0
VO_{2peak}, mL·min^{-1}	2917±557	3099±656	2635±523	2732±539
Body mass index, kg/m²	29.1±4.4	28.5±4.7	29.2±3.6	29.0±4.1
Waist, cm	107.0±9.6	100.1±6.7	107.7±11.1	106.9±11.9
Glycosylated hemoglobin A1c, %	5.4±1.5	5.6±0.4	5.8±1.0	5.8±0.8
Lipid profile				
Total cholesterol, mmol/L	3.6±0.9	3.6±0.7	3.8±0.9	3.9±0.9
Low-density lipoprotein cholesterol, mmol/L	2.1±0.8	1.9±0.6	2.2±0.8	2.2±0.6
High-density lipoprotein cholesterol, mmol/L	1.1±0.3	1.2±0.4	1.0±0.2	1.1±0.2
Triglycerides, mmol/L	1.3±0.6	1.1±0.3	1.7±0.8	1.8±1.9
ApoA1, g/L	1.3±0.2	1.4±0.2	1.2±0.2	1.4±0.2
ApoB, g/L	0.7±0.2	0.7±0.2	0.8±0.2	0.8±0.2

Values are mean±SDs. Apo indicates apolipoprotein; and VO_{2peak}, peak oxygen uptake. HIIT indicates high intensity interval training.

Table 3. Results for the Main Outcome ΔmaxLCBI$_{4mm}$ and Secondary Outcomes

	Baseline to follow-up Exercise group HIIT(n=20)	Baseline to follow-up Control group (n=29)	Group difference at follow-up HIIT vs control group
ΔMaxLCBI$_{4mm}$	−47.7 (−100.3 to 5.0) *P*=0.075	−46.5 (−87.5 to −5.4) *P*=0.027	−1.2 (−65.8 to 63.4) *P*=0.970
VO_{2peak}, mL·kg^{-1}·min^{-1}	3.1 (1.5 to 4.7) *P*<0.001	1.0 (−0.2 to 2.2) *P*=0.113	2.2 (0.2 to 4.1) *P*=0.034
Body mass index, kg/m^2	−0.8 (−1.3 to −0.3) *P*=0.004	0.0 (−0.4 to 0.4) *P*=0.887	−0.7 (−1.4 to −0.1) *P*=0.027
Waist, cm	−3.5 (−5.1 to −2.0) *P*<0.001	−0.9 (−2.0 to 0.1) *P*=0.083	−2.6 (−4.4 to −0.7) *P*=0.008
Glycosylated hemoglobin A1c, %	0.1 (−0.3 to 0.5) *P*=0.578	0.1 (−0.2 to 0.5) *P*=0.485	0.0 (−0.5 to 0.5) *P*=0.993
Total cholesterol, mmol/L	−0.1 (−0.4 to 0.3) *P*=0.725	0.1 (−0.2 to 0.3) *P*=0.639	−0.1 (−0.5 to 0.3) *P*=0.555
Low-density lipoprotein cholesterol, mmol/L	−0.1 (−0.4 to 0.2) *P*=0.412	0.0 (−0.2 to 0.20) *P*=0.981	−0.1 (−0.4 to 0.2) *P*=0.516
High-density lipoprotein cholesterol, mmol/L	0.2 (0.1 to 0.2) *P*<0.001	0.1 (0.0 to 0.1) *P*=0.010	0.1 (0.0 to 0.2) *P*=0.040
Triglycerides, mmol/L	−0.3 (−0.8 to 0.2) *P*=0.223	0.2 (−0.2 to 0.6) *P*=0.265	−0.5 (−1.1 to 0.1) *P*=0.081
ApoA1, g/L	0.1 (0.1 to 0.2) *P*<0.001	0.1 (0.0 to 0.1) *P*<0.002	0.0 (0.0 to 0.1) *P*=0.230
ApoB, g/L	0.0 (−0.1 to 0.0) *P*=0.336	0.0 (−0.1 to 0.1) *P*=0.846	0.0 (−0.1 to 0.1) *P*=0.368

Results for the main outcome maxLCBI$_{4mm}$ and secondary outcomes showing treatment effect as time×group interaction with 95% confidence intervals and *P* value for high intensity interval training compared with control. Apo indicates apolipoprotein; HIIT, high intensity interval training; maxLCBI$_{4mm}$, the maximum lipid core burden index within any 4 mm segment across the entire lesion; and VO_{2peak}, peak oxygen uptake.

had similar reduction in maxLCBI$_{4mm}$ as observed in the group receiving intensive statin therapy (40 mg rosuvastatin), and somewhat lower than seen after treatment with proprotein convertase subtilisin/kexin type 9 inhibitors.[16] To reduce the risk of confounding, only patients on stable lipid lowering therapy for more than 6 weeks were included in the CENIT trial. Furthermore, there were no changes in lipid-lowering therapy for any patients during the study period, and none of the patients used other anti-inflammatory therapies that potentially could influence results. It seems that this strategy was successful as during the study period neither low-density lipoprotein cholesterol nor apolipoprotein B changed in either group.

Although it is difficult to isolate the effect of exercise in our study, we argue that our results provide evidence that physical exercise may reduce lipid content in coronary atheromatous plaques and contribute to plaque stabilization. The results in this study support our previous findings with radiofrequency IVUS demonstrating reduced necrotic core in coronary plaques following aerobic exercise with different intensity.[7] However, future studies are needed to confirm our results and to understand why some individuals respond in a way that is highly beneficial in terms of reduced lipid content as a function of a modest increase in VO_{2peak} whereas others do not. Understanding this may lead to a more precise prediction of treatment outcomes, treatment goals and improved individual patient care.

The mechanisms for reduced maxLCBI$_{4mm}$ following improved VO_{2peak} remain unclear. Beneficial effects on established cardiovascular risk factors may be of importance. In our study, high-density lipoprotein cholesterol and apolipoprotein A1 increased significantly in both groups at follow-up and could represent a link between physical exercise and reduced maxLCBI$_{4mm}$. Furthermore, there is strong evidence that coronary plaque progression and characteristics are influenced by local factors in the coronary artery tree.[25] Studies using computational fluid dynamics modeling have linked low wall shear stress to plaque volume progression and high wall shear stress to a more vulnerable plaque phenotype.[26] In our previous study using HIIT as a model of bouts of "systemic" high wall shear stress, we demonstrated that plaque vulnerability could change in either direction when comparing separate lesions in the same coronary artery.[7] This observation supports that local circulatory factors are important for plaque development and that assessing plaque biology following exercise is complex as the potential effect from exercise may influence different coronary segments divergently. In the CENIT trial, which allowed for multivessel NIRS imaging, we compared changes in maxLCBI$_{4mm}$ between different coronary arteries within the same patient and observed a similar phenomenon

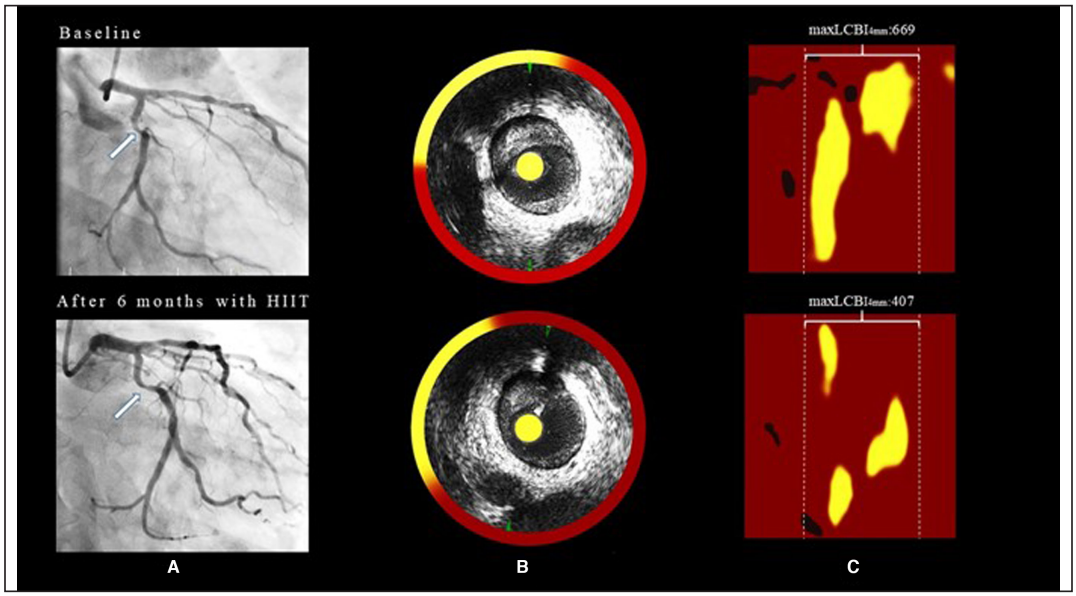

Figure 2. Case demonstrating findings from an imaged vessel with coronary angiography and near-infrared spectroscopy combined with intravascular ultrasound (NIRS-IVUS) in a patient in the high intensity interval training-group at baseline (upper panel) and at follow-up (lower panel).
A, Coronary angiogram with arrow showing a plaque in the proximal segment of the circumflex artery. **B**, Cross-section of NIRS-IVUS image where the yellow circumferential rings represent lipid accumulation within the plaque. **C**, NIRS chemogram demonstrating maxLCBI$_{4mm}$ at baseline and at follow-up with a reduction in maxLCBI$_{4mm}$ from 669 to 407 during the intervention period. Yellow represents high probability of lipid and red denotes no lipid. HIIT indicates high intensity interval training; and maxLCBI$_{4mm}$, maximum lipid core burden index within any 4 mm segment across the entire lesion.

as one third of the patients had plaques where maxLCBI$_{4mm}$ changed in both directions. However, in the majority of plaques with increased maxLCBI$_{4mm}$ during the study period, the baseline maxLCBI$_{4mm}$ was low and the increase modest. Nevertheless, this observation indicates that even for lipid content in coronary atheromatous plaques local circulatory factors may be of significance. Interestingly, studies using coronary computed tomography angiography have demonstrated a higher prevalence of coronary artery calcification and fewer mixed plaques in athletes compared with less active controls.[5,6] Thus, it is suggested that high-volume aerobic endurance exercise may induce a more stable, less inflamed and therefore more benign plaque phenotype compared with sedentary counterparts.[5]

Study Limitations

Several limitations should be taken into consideration when interpreting the CENIT trial. The sample size was small. However, the study protocol with invasive procedures means obvious limitations and the number of included patients is higher than in most other exercise studies with a similar design. The number of lipid-rich plaques was lower than expected in baseline acquisitions and the power problem was reinforced as 11 participants had a maxLCBI$_{4mm}$ <100 at baseline and thus were excluded from further analyses. Furthermore, this phenomenon appeared imbalanced between the 2 groups.

Like other exercise trials with equivalent design, we experienced a challenge with respect to the level of exercise and activity in the control group, as these patients were given advice to follow recommendation in guidelines including principles for cardiac rehabilitation following PCI. Thus, the intended difference in exercise level between patients randomized to HIIT and the control group may have been diminished. The plaque with the highest maxLCBI$_{4mm}$ at baseline was located in stented artery in 16 of 49 patients, but only 6 plaques located distally to an implanted stent were included in the analyses. Thus, it is unlikely that altered flow dynamics following stent implantation has influenced our results significantly. The positive correlation demonstrated between increase in VO_{2peak} and reduction in lipid contents and the larger reduction in maxLCBI$_{4mm}$ observed in patients with an increase in VO_{2peak} above 1 MET were results of a secondary post hoc

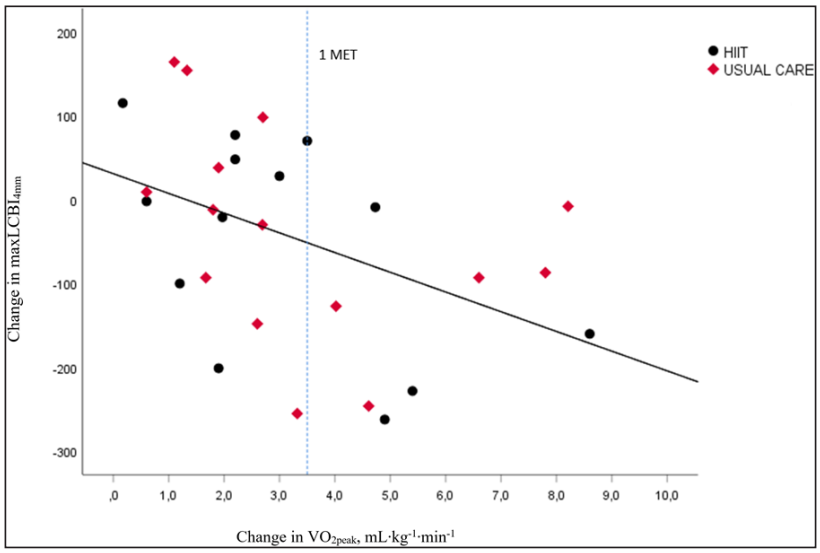

Figure 3. Scatterplot between ΔVO_{2peak} and Δ during the intervention period in patients with increased VO_{2peak} (Spearman's correlation −0.44, *P*=0.009).
HIIT indicates high intensity interval training; $maxLCBI_{4mm}$, the maximum lipid core burden index within any 4 mm segment across the entire lesion; and VO_{2peak}, peak oxygen uptake.

analysis and accordingly only hypothesis generating. A strength of our study is that patients were on stable lipid lowering drugs for at least 6 weeks before inclusion with no change in this medication throughout the study. Furthermore, NIRS data were analyzed at an independent core laboratory and validated exercise protocols in line with contemporary guidelines were used.

CONCLUSIONS

In the CENIT trial, we did not demonstrate a significant difference in $maxLCBI_{4mm}$ between 6 months of supervised HIIT versus usual care following PCI for stable CAD. Lipid content was numerically reduced in both groups with a slightly stronger evidence of a difference in the control group. A significant, moderate positive correlation was demonstrated between increase in VO_{2peak} and reduction in lipid content suggesting that exercise with a subsequent increase in fitness may reduce lipid content in coronary atheromatous plaques.

ARTICLE INFORMATION

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Disclosures

None.

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PAPER II

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FULL RESEARCH PAPER
Coronary artery disease

High-intensity interval training induces beneficial effects on coronary atheromatous plaques: a randomized trial

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Aims Coronary atheroma volume is associated with risk of coronary events in coronary artery disease (CAD). Exercise training is a cornerstone in primary and secondary prevention of CAD, but the effect of exercise on coronary atheromatous plaques is largely unknown. We assessed the effect of 6 months supervised high-intensity interval training (HIIT) on coronary plaque geometry using intravascular ultrasound in patients with stable CAD following percutaneous coronary intervention (PCI).

Methods and results Sixty patients were randomized to two sessions of weekly supervised HIIT at 85–95% of peak heart rate ($n = 30$) or to follow contemporary preventive guidelines (control group, $n = 30$). The study endpoints were change in percent atheroma volume (PAV) and total atheroma volume (TAV) normalized for segment length (TAV_{norm}) at 6-month follow-up. The change in average PAV for matched coronary segments from baseline to follow-up showed a significant between-group difference (−1.4, 95% CI: −2.7 to −0.1, $P = 0.036$). There was a significant reduction in the HIIT group (−1.2, 95% CI: −2.1 to −0.2, $P = 0.017$) while not in the control group (0.2, 95% CI: −0.7 to 1.1, $P = 0.616$). TAV_{norm} was reduced (−9 mm^3, 95% CI: −14.7 to −3.4, $P = 0.002$) after HIIT, with a significant between-group difference (−12.0 mm^3, 95% CI: −19.9 to −4.2, $P = 0.003$).

Conclusion In patients with established CAD, a regression of atheroma volume was observed in those undergoing 6 months of supervised HIIT compared with patients following contemporary preventive guidelines. Our study indicates that HIIT counteracts atherosclerotic coronary disease progression and reduces atheroma volume in residual coronary atheromatous plaques following PCI.

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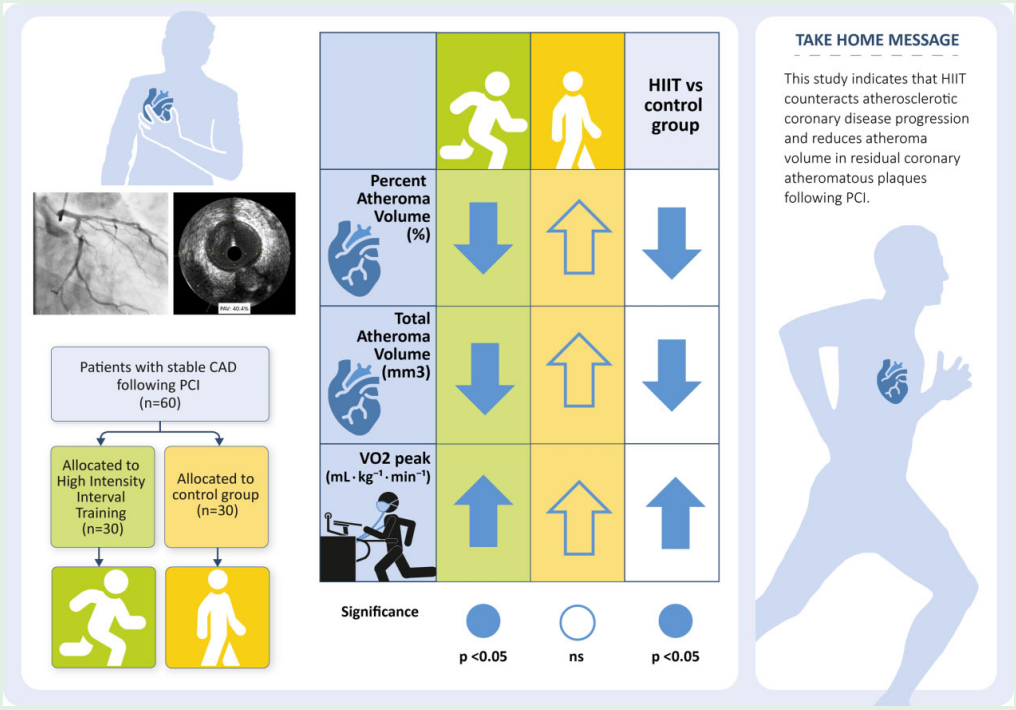
Trial registration ClinicalTrials.gov NCT02494947.

Graphical Abstract



Keywords Physical exercise • High-intensity interval training • Coronary atheromatous plaques • Atheroma volume • Intravascular ultrasound

Clinical perspective

What's new?

- Physical exercise following percutaneous coronary intervention in patients with stable coronary artery disease reduced coronary atheroma volume in patients following high-intensity interval training compared with patients following contemporary preventive guidelines

What are the clinical implications?

- This study indicates that high-intensity interval training counteracts atherosclerotic coronary disease progression and reduces atheroma volume in residual coronary atheromatous plaques following PCI.
- Our results reinforce the scientific background for recommending physical exercise as an important component of cardiac rehabilitation programmes.

Introduction

A multitude of beneficial biological effects are demonstrated with regular physical exercise.[1] A meta-analysis from 2016 demonstrated that exercise-based cardiac rehabilitation reduced cardiovascular mortality for patients hospitalized for coronary artery disease (CAD),[2] and physical activity is well recognized as an important and integral part of cardiac rehabilitation programmes. Coronary atheromatous plaque volume and composition are both strongly related to the risk of acute and recurrent coronary events and outcomes.[3–5] The association between coronary plaque morphology and future cardiac risk is demonstrated in several trials using novel intracoronary imaging techniques.[6–10] In the Providing Regional Observations to Study Predictors of Events in the Coronary Tree (PROSPECT 2) trial, plaque burden and plaque lipid content were both predictors for future cardiac events.[11] However, the effects of physical exercise on coronary atheromatous plaques remain largely unknown. Coronary computed angiography studies on athletes have revealed a higher distribution of dense and more abundant coronary artery calcification whilst CAD in sedentary patients shows more mixed plaque morphologies. The clinical aspect of these findings are highly uncertain, but the hypothesis that exercise induces beneficial effects on plaque remodelling to generate more calcific lesions that are less likely to destabilize, has been strengthened.[12,13] Using radiofrequency intravascular ultrasound (IVUS), we have previously demonstrated a reduction in necrotic core after a period of exercise training in patients with CAD.[14] In the Impact of Cardiac Exercise Training in Coronary Atheromatous Plaques Evaluated by Near-Infrared Spectroscopy (CENIT) trial,[15] we observed that 6 months of high-intensity interval training (HIIT) did not reduce lipid content in coronary plaques compared with usual care. However, a moderate negative correlation between change in peak oxygen uptake (VO_{2peak}) and change in lipid content was demonstrated. In the present

article, results from the analyses of the predefined secondary endpoints assessing atheroma volumes using grey-scale IVUS are presented. We hypothesized that HIIT would induce beneficial effects on coronary atheromatous plaque volumes, i.e. a reduction in percent- and total atheroma volume, compared with patients following contemporary preventive guidelines.

Methods

Study sample and design

The CENIT was an investigator-initiated, single centre, open, parallel, randomized controlled trial conducted at St. Olavs University Hospital in Trondheim, Norway (ClinicalTrials.gov: NCT02494947). The study complied with the Declaration of Helsinki and was approved by the Regional Ethics Committee of Central Norway (2015/210).

All patients gave written informed consent before entering the study. Patients with stable angina undergoing percutaneous coronary intervention (PCI) were eligible for inclusion. Lipid-lowering therapy had to be used for at least 6 weeks before inclusion in the study. Exclusion criteria were previous coronary artery bypass surgery, known inflammatory disease other than atherosclerosis, planned surgery within the next 6 months, inclusion in another randomized trial or inability to comply with the study protocol due to any somatic disease, physical disability, mental problems, or already performing physical activity at a similar or higher level than the prescribed activity for the intervention group. Following successful PCI of all haemodynamic significant stenoses with implantation of drug-eluting stents, multivessel intravascular imaging was performed when feasible by a combination IVUS- and near-infrared spectroscopy (NIRS) catheter using an automated pullback system (TVC-MC8 model system with a 3.2Fr 40 MHz catheter, Infraredx, Burlington, Massachusetts). Following administration of intracoronary nitroglycerine, the NIRS-IVUS catheter was positioned as distally as possible in the coronary artery. To ensure matching coronary segments at baseline and follow-up, the distal starting points of the pullbacks were recorded angiographically to assist in registration of the corresponding segments at follow-up.

Following PCI and intracoronary imaging, all patients received guideline-directed medical therapy. The study protocol allowed no adjustments of lipid-lowering therapy throughout the study. All patients were at the time of discharge given general advice to maintain a healthy lifestyle including diet, smoking cessation, daily use of statins and anti-platelet drugs, achieve a blood pressure <140/90 mmHg, HbA1c values < 53 mmol/mol (7%) and to perform regular exercise.[16] They were also encouraged to participate in local cardiac rehabilitation programmes and heart schools.

After complete baseline acquisitions and with the use of a web-based randomization system, patients underwent randomly allocation assigned in random block sizes to either an intervention with HIIT or a control group (*Figure 1*).

Intervention

The HIIT intervention consisted of two sessions weekly of supervised endurance training mainly on treadmills and bicycles. Each session started with a 10 min warm-up at moderate intensity (60–70% of peak heart rate, HR_{peak}), followed by 4 × 4 min intervals with an intensity at 85–95% of HR_{peak} with 3 min of active recovery at moderate intensity between intervals, ending with a 5 min cool down period.[17,18] All exercise sessions were supervised by physiotherapists with experience in cardiac rehabilitation. The HIIT group was also encouraged to perform home-based endurance training with sessions of interval training, and any activity mode involving dynamic work with large muscle groups was recommended. In order to guide exercise intensity, increase motivation and adherence to home-based exercise, patients in the HIIT group were provided with a wearable device for HR monitoring and activity tracking (Mio Slice, Mio Global, Toronto, Canada). The control group was advised to follow contemporary preventive guidelines[19] but did not receive any supervision nor wearable device for physical activity tracking, throughout the study.

Imaging analysis

Anonymous angiograms and intravascular ultrasound data were analysed at an independent core laboratory (KCRI, Krakow, Poland) without any knowledge of patient characteristics or randomization allocation. IVUS analyses of all cross-sectional images and quantitative measurements were performed using CASS IntraVascular 2.1 software, Pie Medical Imaging, BV. The matched segments of corresponding regions of interest at baseline and follow-up were identified using fiduciary points such as side branches and the implanted stent, as well as comparison of angiographic records, and analyzed according to consensus standards.[20,21] Segments not represented at both baseline and follow-up were excluded. The regions of interest were defined as a target segment lesion with an atherosclerotic plaque compromising the lumen by at least 30% by cross-sectional area, with the 5 mm segments proximal and distal to the target segment as reference.

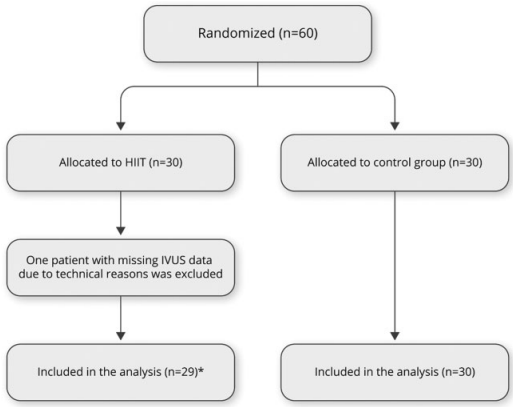


Figure 1 Flow chart illustrating enrolment, randomization, allocation, and follow-up throughout the study. In one patient, the pullback acquisition was not interpretable, and the patient was excluded from the analyses. *At 6 months follow-up, one patient had emigrated, and one patient underwent coronary artery bypass grafting, but they were included in the analyses. HIIT, high-intensity interval training; IVUS, intravascular ultrasound.

The vessel with the highest maximum plaque burden at baseline was defined as the target vessel.

Two IVUS-derived efficacy measures reflecting plaque geometry was calculated. In every cross-sectional image selected for analysis, the lumen membrane cross-section area (Lumen $_{CSA}$) and external elastic membrane cross-section area (EEM $_{CSA}$) leading edges were defined by manual planimetry.[22] The total atheroma area was determined as the area between these leading edges: total atheroma area = EEM $_{CSA}$—Lumen $_{CSA}$. Total atheroma volume (TAV) was calculated based on the pullback speed during image acquisition. TAV was normalized to account for differences in segment lengths between patients: $TAV_{norm} = [\sum$ (EEM $_{CSA}$—Lumen $_{CSA}$)/segment length] × median segment length in the population. Percent atheroma volume (PAV) was calculated using the following equation: PAV = [$\sum$ (EEM $_{CSA}$—Lumen $_{CSA}$)/$\sum$EEM $_{CSA}$] · 100

Minimum lumen area (MLA) was defined as the smallest area through the centre point of the lumen throughout the region of interest. Remodelling was assessed by means of the remodelling index (RI), expressed as the EEM $_{CSA}$ at the MLA site divided by the reference EEM $_{CSA}$. Positive remodelling was defined as RI ≥ 1.05 and negative remodelling as RI ≤ 0.95. Values in between were considered neutral (no remodelling).

Cardiovascular risk factors and exercise testing

Medical history uptake, physical examination, anthropometrics, fasting blood sampling, and echocardiography were performed at baseline and follow-up. Two weeks following PCI, a cardiopulmonary exercise test (CPET) was performed to determine exercise tolerance, VO_{2peak} and HR $_{peak}$ in order to tailor exercise prescription and evaluate training effects (Jaeger® Vyntus CPX, Hoechber, Germany).[17] Data from the 12-lead electrocardiogram, blood pressure, and respiratory gases were recorded continuously. The individualized ramp protocol started at a speed and inclination set during the 15 min warm-up guided by the Borg scale. Thereafter workload was increased either by 1 km/h in speed or by 2% incline every minute until the participant stopped the test due to exertion, or VO_{2max} was achieved. VO_{2max} was defined as a flattening of the oxygen uptake curve despite an increased workload combined with a respiratory exchange ratio above 1.05. The protocol lasted 8–12 min. After the study period the patients were readmitted to the hospital for follow-up CPET, echocardiography, blood sampling, and intracoronary imaging procedures.

Outcomes

In this paper, we present the results from the analyses of the predefined secondary endpoints assessing atheroma volumes using grey-scale intravascular ultrasound in the CENIT population. The aim of this study was to compare the mean change in PAV and TAV_{norm} from baseline to 6 months follow-up between the HIIT group and the control group. Other outcome measures were the mean change in RI, MLA, VO_{2peak}, body mass index (BMI), waist circumference, blood lipids including cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, Apolipoprotein A, Apolipoprotein B, and glycosylated haemoglobin A1c (HbA1c).

Statistical analyses

The primary endpoint in the CENIT trial was $maxLCBI_{4mm}$. However, we had no data on the effect of physical exercise on $maxLCBI_{4mm}$ when planning the study. In a previous study using radiofrequency IVUS of 36 patients undergoing PCI, we demonstrated a reduction in necrotic core both for patients undertaking 12 weeks of HIIT and those exercising at moderate intensity with no in-between group difference.[14] Based on this experience, we designed the CENIT study with a larger sample size and a prolonged intervention period to increase the statistical power with regard to the primary endpoint.

The main study endpoints were tested using linear mixed models with time and intervention group (HIIT or control) as fixed effects variables. Dependence of observations within individuals was handled by including individual-specific random intercepts. Due to randomization, mean outcomes at baseline were in the models constrained to be equal between the groups. Similar models were used for the other outcome measurements. Baseline clinical characteristics are presented as means with standard deviations or as frequencies and percentages. Normality of residuals was checked by visual inspection of normal QQ-plots. Normally distributed continuous variables were compared using the Student *t*-test and categorical variables were compared by the Chi-square test or Fisher's exact test. *P*-values <0.05 were considered statistically significant and confidence intervals (CIs) are presented at the 95% level. No formal adjustment for multiple testing was performed. All principal analyses were done in the intention-to-treat population, consisting of all randomized patients regardless of treatment received. All statistical analyses were performed with SPSS, version 26.0 (IBM corporation) and Microsoft Office Excel 2016.

Results

Study patients

From February 2016 to April 2019, 60 patients were enrolled in the study. One patient in the HIIT group had unsuccessful intracoronary imaging and was excluded from the analyses. Thus, the study population consisted of 59 patients (29 in the HIIT group and 30 in the control group). One patient developed severe angina pectoris and underwent coronary artery bypass grafting and another patient emigrated, leaving 57 patients at follow-up (*Figure 1*). However, all 59 patients were included in the intention-to-treat analyses. Baseline clinical features, laboratory measures and lesion characteristics of the randomized population were well matched and similar in most respect (*Table 1*).

Lesion characteristics

At baseline, coronary atheromatous lesions in the target artery had on average a maximal PAV of 66 (SD 12) % in the HIIT group vs. 67 (SD 8) % in the control group, and the mean MLA was 4.3 (SD 1.6) mm vs. 4.7 (SD 1.3) mm, respectively. Dual anti-platelet therapy (most commonly aspirin and clopidogrel) was administered in all patients. Noteworthy, there were no adjustments in lipid-lowering therapy during the study period. The results from linear mixed models analyses regarding the change in coronary plaque geometry from baseline to follow-up are presented in *Table 2*. When comparing the change in average PAV for matched coronary segments from baseline to follow-up, a statistically significant between-group difference (−1.4, 95% CI: −2.7 to −0.1, $P = 0.036$) was demonstrated. There was a significant reduction in average PAV in the HIIT group (−1.2, 95% CI: −2.1 to—0.2, $P = 0.017$) while not in the control group (0.2, 95% CI: −0.7 to 1.1, $P = 0.616$). TAV_{norm} was reduced by 9.0 mm^3 (95% CI:14.7 to 3.4, $P = 0.002$) after HIIT compared to a non-significant increase of 3.0 mm^3 (95% CI: −2.4 to 8.4, $P = 0.268$) in the control group, resulting in a between-group difference of 12.0 mm^3 (95% CI:(−19.9 to −4.2, $P = 0.003$).

There was a statistically significantly larger reduction in RI the HIIT group compared to the control group, with a significant change in the HIIT group only. There were no statistically significant differences or changes in MLA during the study.

Cardiovascular risk factors

The results from linear mixed model analysis regarding clinical and biochemical outcomes are presented in *Table 3*. VO_{2peak} improved more after HIIT than in the control group, with a significant difference in the HIIT group only. There was also a larger reduction in BMI, and waist circumference in the HIIT group compared with the control group. In both groups, a modest increase was observed for both HDL cholesterol and ApoA1, with no evidence of between-group differences. Otherwise, there were no significant differences or changes at follow-up in biochemical variables (*Table 3*). The IVUS recordings were performed without complications. One patient in the control group suffered a minor stroke at the end of the follow-up period, otherwise there were no adverse events during the study.

Table 1 Baseline clinical and laboratory characteristics of the randomized population

	Exercise group HIIT ($n = 29$)	Control group ($n = 30$)	*P*-value
Characteristics			
Age, years	57.3 ± 6.8	58.7 ± 7.4	0.47
No of males/females	27/2	27/3	
Body mass index, kg/m^2	28.9 ± 4.0	29.1 ± 3.5	0.77
Medical history, *n* (%)			
Hypertension, medically treated	16 (55)	16 (53)	0.89
Diabetes mellitus, medically treated	2 (7)	5 (17)	0.25
Smoking currently	1 (4)	4 (13)	0.17
Smoked previously	16 (55)	16 (53)	0.89
Prior history of coronary artery disease	11 (38)	13 (43)	0.67
Congestive heart failure	1 (4)	2 (7)	0.58
Left ventricular ejection fraction, %	53 ± 3	52 ± 3	0.09
Medication, *n* (%)			
Dual anti-platelet therapy	29 (100)	30 (100)	
High potent statins	21(72)	26 (87)	0.17
Low potent statins	8 (28)	4 (13)	0.17
Combined therapy with ezetimib	2 (7)	3 (10)	0.67
β-Blockers	10 (35)	11 (37)	0.86
Angiotensin-converting enzyme inhibitors/angiotensin II receptor antagonists	16 (55)	14 (47)	0.51
Laboratory measures			
Total cholesterol, mmol/L	3.7 ± 0.8	3.8 ± 0.9	0.46
High-density lipoprotein, mmol/L	1.1 ± 0.3	1.0 ± 0.2	0.17
Low-density lipoprotein, mmol/L	2.1 ± 0.7	2.2 ± 0.7	0.52
Triglycerides, mmol/L	1.3 ± 0.6	1.6 ± 0.8	0.11
Apolipoprotein A1, g/L	1.3 ± 0.2	1.3 ± 0.2	0.38
Apolipoprotein B, g/L	0.7 ± 0.2	0.8 ± 0.2	0.47
Haemoglobin, g/L	14.7 ± 1.4	14.3 ± 1.8	0.29
Serum creatinine, mg/dL	77.3 ± 13.6	81.0 ± 13.0	0.29
Glycosylated haemoglobin, %	5.6 ± 0.8	5.8 ± 1.0	0.52
Angiographic findings			
Coronary artery lesion location, *n* (%)			
LAD	16 (55)	14 (47)	
Cx	6 (21)	5 (17)	
RCA	7 (24)	9 (30)	
Intermediate	0	2 (7)	

Values are presented as mean ± SD for continuous variables and as frequencies (%) for categorical variables. Results from Student *t*-tests and chi-square tests.
High potent statin: atorvastatin 80/40 mg, simvastatin 80/40 mg, rosuvastatin 40/20 mg
Low potent statin: atorvastatin 20/10 mg, simvastatin 20/10 mg, rosuvastatin 10/5 mg, pravastatin 40mg
HIIT, high-intensity interval training; LAD, left anterior descending artery; Cx, circumflexa; RCA, right coronary artery.

Discussion

In this trial, where 6 months of HIIT significantly increased VO_{2peak}, a reduction in atheroma volume in residual coronary atherosclerosis was observed in patients with stable angina undergoing PCI. PAV and TAV both showed significantly larger atheroma volume regression in the HIIT group compared with usual care. To our knowledge, this is the largest randomized clinical trial using intravascular ultrasound to evaluate the potential anti-atherogenic effects of intensive exercise, and the first one to demonstrate a significant effect of exercise on plaque geometry.

Although it has been hypothesized that exercise training may have anti-atherogenic effects, strong evidence of this has not been demonstrated in humans. Only a few previous studies have assessed potential effects of exercise on coronary atheromatous plaques using intravascular ultrasound. In 2010, Sixt *et al.*[23] failed to demonstrate an effect of a multifactorial intervention programme on coronary plaque burden. In a previous study, our group compared HIIT vs. moderate continuous exercise and found a modest reduction in coronary necrotic core in both exercise groups.[14] These findings were essentially reproduced in a small study by Nishitani-Yokoyama M, *et al.*[24], demonstrating a correlation between exercise volume and a reduction in plaque and lipid volume. In the current study, we found significant reductions in PAV (−1.2%) and TAV (−9.0mm3) in patients undergoing HIIT compared with usual care. The primary endpoint in the CENIT trial was change in lipid content measured as maximum lipid core

Table 2 **Outcomes assessed by intravascular ultrasound**

	Exercise group HIIT($n=29$)		Control group ($n=30$)		Change from baseline to follow-up		Group difference at follow-up
	Baseline	Follow-up	Baseline	Follow-up	Exercise group	Control group	HIIT vs. control group
Max PAV (%)	66.3 ± 12.1	65.3 ± 10.2	67.2 ± 8.4	68.6 ± 9.6	−1.1 (−2.7 to 0.5) *P = 0.170*	1.4 (−0.1 to 2.9) *P = 0.072*	−2.5 (−4.6 to −0.3) *P = 0.025*
Average PAV (%)	49.5 ± 9.4	48.3 ± 8.4	48.6 ± 6.1	48.9 ± 6.0	−1.2 (−2.1 to −0.2) *P = 0.017*	0.2 (−0.7 to 1.1) *P = 0.616*	−1.4 (−2.7 to −0.1) *P = 0.036*
TAV_{norm} (mm^3)	162.7 ± 60.5	154.9 ± 57.2	179.4 ± 61.4	182.1 ± 63.3	−9.0 (−14.7 to −3.4) *P = 0.002*	3.0 (−2.4 to 8.4) *P = 0.268*	−12.0 (−19.9 to −4.2) *P = 0.003*
Vessel volume (mm^3)	352.9 ± 269.6	336.7 ± 253.1	472.5 ± 255.7	475.6 ± 260.9	−17.1 (−35.3 to 1.2) *P = 0.067*	4.1 (−13.2 to 21.4) *P = 0.638*	−21.2 (−46.3 to 4.0) *P = 0.097*
Lumen volume (mm^3)	165.0 ± 98.0	163.1 ± 96.7	242.9 ± 138.2	242.4 ± 137.6	−2.5 (−11.4 to 6.4) *P = 0.573*	0.1 (−8.3 to 8.6) *P = 0.973*	−2.7 (−14.9 to 9.5) *P = 0.665*
Atheroma volume (mm^3)	187.9 ± 179.7	173.6 ± 162.8	229.6 ± 124.5	233.2 ± 130.3	−14.7 (−26.5 to −2.9) *P = 0.016*	4.1 (−7.11 to 15.3) *P = 0.467*	−18.8 (−35.0 to −2.5) *P = 0.024*
Lesion length (mm)	24.2 ± 15.1	23.8 ± 2.8	29.5 ± 12.9	29.5 ± 2.4	−0.7 (−1.7 to 0.3) *P = 0.164*	0.0 (−0.9 to 1.0) *P = 0.974*	−0.7 (−2.1 to 0.7) *P = 0.299*
MLA (mm^2)	4.3 ± 1.6	4.3 ± 1.6	4.7 ± 1.3	4.4 ± 1.4	−0.1 (−0.4 to 0.2) *P = 0.454*	−0.2 (−0.5 to 0.1) *P = 0.152*	*0.1* (−0.3 to 0.5) *P = 0.646*
Remodelling index	0.9 ± 0.2	0.8 ± 0.2	0.9 ± 0.1	0.9 ± 0.2	−0.1 (−0.1 to 0.0) *p < 0.001*	0.0 (−0.1 to 0.0) *P = 0.633*	−0.1 (−0.1 to 0.0) *P = 0.026*

Values are mean ± SD for continuous variables. Results from linear mixed models for main outcome and other IVUS measurements. Outcomes showing treatment effect as time × group interaction with 95% confidence intervals and *P*-value for high-intensity interval training compared with control. PAV, percent atheroma volume; TAV, total atheroma volume; MLA, minimal lumen area

Table 3 Clinical and biochemical outcomes

	Exercise group HIIT($n=29$)		Control group ($n=30$)		Change from baseline to follow-up		Group difference at follow-up
	Baseline	Follow-up	Baseline	Follow-up	Exercise Group	Control group	HIIT vs. control group
VO_{2peak}, mL·/kg·/min	32.6 ± 5.8	35.9 ± 6.6	29.0 ± 6.1	30.5 ± 6.9	2.7 (1.5 to 3.9)	1.0 (−0.1 to 2.1)	1.7 (0.1 to 3.3)
					$P<0.001$	$P=0.078$	$P=0.039$
Waist, cm	105.5 ± 8.9	100.2 ± 6.8	107.6 ± 10.9	106.8 ± 11.7	−3.1 (−4.4 to −1.7)	−0.9 (−2.1 to 0.3)	−2.2 (−4.0 to −0.4)
					$P<0.001$	$P=0.145$	$P=0.021$
Body mass index, kg/m^2	29.0 ± 4.0	28.3 ± 4.2	29.1 ± 3.5	29.0 ± 4.0	−0.7(−1.1 to −0.3)	0.0(−0.4 to 0.3)	−0.7(−1.2 to −0.1)
					$P=0.001$	$P=0.830$	$P=0.021$
Glycosylated haemoglobin A1c, %	5.6 ± 0.8	5.5 ± 0.6	5.8 ± 1.0	5.8 ± 0.8	−0.2 (−0.3 to 0.3)	0.05 (−0.1 to 0.2)	−0.2 (−0.5 to 0.4)
					$P=0.100$	$P=0.559$	$P=0.101$
Total cholesterol, mmol/L	3.7 ± 0.8	3.8 ± 0.8	3.8 ± 0.9	3.9 ± 0.9	0.0 (−0.2 to 0.3)	0.1 (−0.2 to 0.3)	0.0 (−0.4 to 0.3)
					$P=0.845$	$P=0.580$	$P=0.801$
LDL cholesterol, mmol/L	2.1 ± 0.7	2.1 ± 0.7	2.2 ± 0.7	2.2 ± 0.6	−0.1 (−0.3 to 0.1)	0.0 (−0.2 to 0.20)	−0.1 (−0.3 to 0.2)
					$P=0.295$	$P=0.942$	$P=0.462$
HDL cholesterol, mmol/L	1.1 ± 0.3	1.2 ± 0.3	1.0 ± 0.2	1.1 ± 0.2	0.1 (0.1 to 0.2)	0.1 (0.0 to 0.1)	0.1 (0.0 to 0.1)
					$P<0.001$	$P=0.005$	$P=0.074$
Triglycerides, mmol/L	1.3 ± 0.6	1.2 ± 0.4	1.6 ± 0.8	1.8 ± 1.9	−0.2 (−0.6 to 0.2)	0.2 (−0.1 to 0.6)	−0.4 (−0.9 to 0.1)
					$P=0.275$	$P=0.217$	$P=0.081$
ApoA1, g/L	1.3 ± 0.2	1.4 ± 0.2	1.3 ± 0.2	1.4 ± 0.2	0.1 (0.1 to 0.2)	0.1 (0.0 to 0.1)	0.1 (0.0 to 0.1)
					$P<0.001$	$P<0.002$	$P=0.065$
Apo-B, g/L	0.7 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.0 (−0.1 to 0.0)	0.0 (−0.1 to 0.1)	0.0 (−0.1 to 0.0)
					$P=0.259$	$P=0.893$	$P=0.346$

Values are mean ± standard deviations for continuous variables. Results from linear mixed models for the clinical and biochemical outcomes showing treatment effect as time × group interaction with 95% confidence intervals and *P*-value for high-intensity interval training compared with control. VO_{2peak}, peak oxygen uptake; Apo, apolipoprotein

burden index at 4 mm ($maxLCBI_{4mm}$).[15] $MaxLCBI_{4mm}$ decreased significantly in both groups during exercise with no between-group differences, a result that may have different explanations. First, the exclusion of 11 patients with low intracoronary lipid content at baseline amplified a power problem with respect to the low number of patients in the trial. This was supported by a secondary analysis showing a significant correlation between the change in lipid content of atheromatous plaques and an increase in VO_{2peak} during the intervention period, advocating the hypothesis that exercise with high intensity reduces intracoronary lipid content. Second, it may be hypothesized that HIIT induces different effects in coronary atheromatous plaques with respect to atheroma volume and lipid content.

The observed reductions in PAV and TAV demonstrated after 6 months of HIIT is of importance and is comparable to the results of previous statin trials. In the Study of Coronary Atheroma by Intravascular Ultrasound: Effect of Rosuvastatin vs. Atorvastatin (SATURN) study, a 0.99% reduction of PAV with atorvastatin and a 1.22% reduction in PAV with rosuvastatin was demonstrated.[25] However, in a sub study from the Reduction in Yellow Plaque by Aggressive Lipid-Lowering Therapy (YELLOW) Trial, an overall increase in PAV was demonstrated at follow-up in patients on intensive statin therapy, with some evidence of a non-significant reduction in PAV in patients with PAV ≥ 70% at baseline.[26] In a recent study, the effect on PAV of adding the proprotein convertase subtilisin kexin type 9 (PCSK9) inhibitor alirocumab to statin therapy with 20 mg rosuvastatin was assessed, demonstrating a 2.13% reduction in PAV in the intervention group compared with a 0.92% reduction in controls.[27] Accordingly, the plaque volume regression observed after HIIT in the present study is slightly larger than what is observed with statin interventions but less than the demonstrated PAV reduction when adding a PCSK9-inhibitor. A recent meta-regression analysis of 17 prospective pharmacotherapy studies found that each 1% reduction in PAV was associated with a 20% reduction in the odds of major adverse cardiovascular events.[28] Whether HIIT after PCI-treatment of CAD translates into a prognostic advantage remains to be elucidated.

The beneficial effect of physical activity in CAD has been known for decades, but the mechanism by which physical activity potentially protects and affects coronary atherosclerotic plaques remains incompletely understood. The mechanisms underlying the observed atheroma volume reduction in our study are also uncertain and may include both modification of coronary risk factors and a local and direct effect on coronary atherosclerosis. There is strong and consistent evidence that LDL cholesterol is an obligate and causal factor of atherosclerotic cardiovascular disease.[28] The association between plaque regression and a reduction in LDL cholesterol is also documented.[25,29] In our study, LDL cholesterol and Apo-B levels were unchanged. Thus, we have no evidence supporting that the observed reductions in atheroma volume could be explained by a reduction in atherogenic Apo-B particles. Furthermore, a modest increase was observed for both HDL cholesterol and ApoA1 in both groups at follow-up, but no evidence of a between-group difference was found. It is therefore unlikely that the observed increase in HDL cholesterol, potentially enhancing foam cells efflux from coronary atheromatous plaques,[30] is a significant explanatory factor. Moreover, there is increasing evidence that alternations in local haemodynamic forces in the coronary tree are pivotal for the dynamic biology of atheroma progression and regression. In the Prediction of Progression of Coronary Artery Disease and Clinical Outcome Using Vascular Profiling of Shear Stress and Wall Morphology (PREDICTION) study, low endothelial shear stress (ESS) predicted a progression in PAV.[5] These findings were supported by

Eshtehardi et al.[31] demonstrating that coronary segments with PAV ≥ 40% and low ESS had significantly greater increase in plaque area compared to coronary segments without low ESS. Computational fluid dynamics models were not available in our study. Our data supports the hypothesis that HIIT may induce regression of coronary atheroma volume through increased coronary ESS from repeated bouts of increased coronary blood flow.

Study limitations

This study is limited by the small sample size. However, the study protocol with serial invasive procedures involves indisputable limitations and the number of included patients is higher than in previous exercise studies with a similar design. Also, we did not experience unbalanced study arms as in the first publication from CENIT, as the atheroma volume analysis could be performed in all patients with valid recordings. We also experienced some challenges with respect to the level of exercise prescription as patients in the control group were given advice to follow recommendation in guidelines including principles for cardiac rehabilitation. Thus, the intended difference in exercise level between the two groups may have been diminished, and this may have influenced the between-group differences in atheroma regression. In addition, we acknowledge that the exercise levels obtained in the intervention group may be hard to obtain without ongoing and continuous supervision as offered in our study. A strength of our study is that patients were on stable lipid-lowering drugs for at least 6 weeks prior to inclusion with no change in this medication throughout the study. Furthermore, IVUS data were analysed at an independent core laboratory and validated exercise protocols in line with contemporary guidelines were used.

Conclusion

In patients with established CAD, on stable lipid-lowering therapy, a regression of coronary atheroma volume was observed in patients undergoing 6 months of supervised HIIT compared with patients following contemporary preventive guidelines. Our study indicates that HIIT counteracts atherosclerotic coronary disease progression and reduces atheroma volume in residual coronary atheromatous plaques following PCI.

Authors' contributions

E.K.V., U.W., R.W., and E.M. contributed to the conception and design of the work and E.K.V., R.W., and E.M. drafted the manuscript. R.W., K.H., and H.O.N. offered medical expertise and guidance during the invasive imaging and H.O.N. was responsible for handling the collected imaging data. U.W. and I.-L.A.A. contributed to the conception and design of the exercise intervention and I.-L.A.A. offered expertise and guidance during the CPET. T.F. contributed to the statistical analyses. All authors critically revised the manuscript and approved the final version.

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Conflict of interest: None declared.

Data availability

The data underlying this article will be shared upon request to the corresponding author.

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PAPER III

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The association between circulating lipoprotein subfractions and lipid content in coronary atheromatous plaques assessed by near-infrared spectroscopy

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ABSTRACT

Background: Lipid content in coronary atheromatous plaques, measured by near-infrared spectroscopy (NIRS), can predict the risk of future coronary events. Biomarkers that reflect lipid content in coronary plaques may therefore improve coronary artery disease (CAD) risk assessment.
Purpose: We aimed to investigate the association between circulating lipoprotein subfractions and lipid content in coronary atheromatous plaques in statin-treated patients with stable CAD undergoing percutaneous coronary intervention.
Methods: 56 patients with stable CAD underwent three-vessel imaging with NIRS when feasible. The coronary artery segment with the highest lipid content, defined as the maximum lipid core burden index within any 4 mm length across the entire lesion (maxLCBI$_{4mm}$), was defined as target segment. Lipoprotein subfractions and Lipoprotein a (Lp(a)) were analyzed in fasting serum samples by nuclear magnetic resonance spectroscopy and by standard in-hospital procedures, respectively. Penalized linear regression analyses were used to identify the best predictors of maxLCBI$_{4mm}$. The uncertainty of the lasso estimates was assessed as the percentage presence of a variable in resampled datasets by bootstrapping.
Results: Only modest evidence was found for an association between lipoprotein subfractions and maxLCBI$_{4mm}$. The lipoprotein subfractions with strongest potential as predictors according to the percentage presence in resampled datasets were Lp(a) (78.1 % presence) and free cholesterol in the smallest high-density lipoprotein (HDL) subfractions (74.3 % presence). When including established cardiovascular disease (CVD) risk factors in the regression model, none of the lipoprotein subfractions were considered potential predictors of maxLCBI$_{4mm}$.
Conclusion: In this study, serum levels of Lp(a) and free cholesterol in the smallest HDL subfractions showed the strongest potential as predictors for lipid content in coronary atheromatous plaques. Although the evidence is modest, our study suggests that measurement of lipoprotein subfractions may provide additional information

Abbreviations: CAD, Coronary artery disease; NIRS, Near-infrared spectroscopy; LDL, Low-density lipoprotein; HDL, High-density lipoprotein; LDL-C, Low-density lipoprotein cholesterol; HDL-C, High-density lipoprotein cholesterol; Lp(a), Lipoprotein a; maxLCBI$_{4mm}$, The maximum lipid core burden index within any 4mm length across the entire lesion; PCI, Percutaneous coronary intervention; NIRS-IVUS, Near-infrared spectroscopy intravascular ultrasound; CENIT, The impact of Cardiac Exercise Training on Lipid Content in Coronary Atheromatous Plaques Evaluated by Near-Infrared Spectroscopy Trial; NMR, Nuclear magnetic resonance; Apo-A1, Apolipoprotein A1; Apo-A2, Apolipoprotein A2; Apo-B, Apolipoprotein B; BMI, Body mass index; CVD, Cardiovascular disease; IDL, Intermediate-density lipoprotein; VLDL, Very-low-density lipoprotein.

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with respect to coronary plaque composition compared to traditional lipid measurements, but not in addition to established risk factors. Further and larger studies are needed to assess the potential of circulating lipoprotein subfractions as meaningful biomarkers both for lipid content in coronary atheromatous plaques and as CVD risk markers.

1. Introduction

Lipid accumulation and inflammation in the coronary artery vessel wall are pivotal pathophysiological mechanisms in the development of coronary artery disease (CAD). It is demonstrated that the prognosis in CAD is strongly related to plaque geometry and composition [1]. Coronary plaques with a large lipid-rich core and an overlying thin fibrous cap are particularly vulnerable to rupture [2]. Intracoronary imaging studies using near-infrared spectroscopy (NIRS), a technique that enables identification of lipid content in coronary plaques [3,4], have demonstrated that lipid content can predict the risk of future coronary events [5–8]. NIRS is an invasive and resource-intensive procedure, and most often performed in conjunction with coronary angiography in symptomatic patients. Accordingly, it is of clinical interest to identify non-invasive surrogate biomarkers for lipid content in coronary plaques in order to improve risk stratification and optimal preventive treatment.

Lipoproteins, particularly low-density lipoprotein (LDL) cholesterol (LDL-C), play an essential role in atherosclerotic plaque initiation, progression, and composition [9,10]. The risk for future cardiovascular events increases linearly with aggregated concentration of LDL-C, and the risk is also affected by the lifetime exposure of high concentrations [11]. Nevertheless, many patients with CAD have low cholesterol levels, and even with adequately lipid lowering treatment, the risk of future cardiovascular events remain significant [12–14].

Analysis of lipoprotein subfractions provides information of size, density, concentration, and compositions. It is suggested that small and dense LDL particles on cost of large and buoyant LDL particles result in a less favorable risk profile, and it is also questioned whether all high-density lipoprotein (HDL) subfractions hold atheroprotective properties [15–17]) . Furthermore, of increasing interest is Lipoprotein a (Lp (a)) which is an LDL-like particle with pro-atherosclerotic and pro-inflammatory properties. A causal continuous association between Lp (a) concentration and myocardial infarction has been demonstrated, even at low levels of LDL-cholesterol [18–20]. Measurements of lipoprotein subfractions may therefore improve risk stratification beyond traditional lipid measurements.

Studies assessing the association between circulating lipoproteins and lipid content in coronary atheromatous plaques, measured as the -maximum lipid core burden index within any 4 mm length across the entire lesion (maxLCBI$_{4mm}$), are sparse. To our knowledge, only traditional lipid measurements and Lp(a) have been investigated, with no or weak associations [21–24]. Whether a more detailed lipoprotein subfraction analysis can identify new biomarkers with stronger associations to coronary plaque lipid content is unknown. In the present study, we aimed to investigate the association between circulating lipoprotein subfractions and lipid content in coronary atheromatous plaques, measured as maxLCBI$_{4mm}$ by NIRS, in patients with stable CAD undergoing percutaneous coronary intervention (PCI).

2. Methods

2.1. Study design and ethics

This cross-sectional study was based on baseline data from the *Impact of Cardiac Exercise Training on Lipid Content in Coronary Atheromatous Plaques Evaluated by Near-Infrared Spectroscopy* (CENIT) [25]. The study was approved by the regional ethics committee of central Norway (2015:210), registered at clinicaltrials.gov (NCT02494947), and conducted in accordance with the Declaration of Helsinki. Written and informed consent was obtained from all participants, and their personal information was handled and stored with high security in accordance with laws and regulations.

2.2. Study participants

Patients diagnosed with a hemodynamic significant coronary artery stenosis in at least one epicardial vessel that required PCI were screened for inclusion between February 2016 and April 2019 at St. Olavs Hospital in Trondheim, Norway. Inclusion criteria was stable statin therapy for at least six weeks prior to the angiographic examination to avoid anti-atherosclerotic effects following initiation of high-dose statin therapy and for stabilization of the circulating lipid profile [26,27]. Exclusion criteria were prior coronary artery bypass graft surgery and known inflammatory disease (other than atherosclerosis). A total of 60 eligible patients gave written informed consent to participate in the study.

2.3. Intracoronary imaging

Following stent implantation and intracoronary administration of 200 μg nitroglycerine, three-vessel intracoronary imaging was performed when feasible to quantify lipid content in non-culprit coronary plaques. The near-infrared spectroscopy intravascular ultrasound (NIRS-IVUS) catheter (TVC-MC8 model system with a 3.2Fr 40 MHz catheter, Infraredx, Burlington, MA) was positioned as distal as possible in the coronary artery and pulled back to the ostium or the guiding catheter at a speed of 0.5 mm/s. Intracoronary imaging data and angiograms were analyzed with a commercial software (Pie Medical Imaging Software, CAAS Intravascular) at an independent core facility (KCRO, Krakow, Poland) blinded for patient characteristics. The stented segment with its corresponding 5 mm edge segments in both directions was excluded from the analysis.

The lipid core burden index ranges from 0 to 1000 and was calculated from a NIRS derived chemogram with color coded pixels (Fig. 1). The colors span from red to yellow with increased probability of lipid-rich plaques [3]. The coronary artery segment with the highest measured lipid content, defined as the maximum lipid core burden index (range between 0 and 1000) within any 4 mm segment length across the entire lesion (maxLCBI$_{4mm}$), was considered the most diseased segment and thus defined as target segment.

2.4. Data collection

Following standard in-hospital procedures, fasting venous blood samples were collected early in the morning the day after PCI. Within 1 hour, a 5 mL serum tube with clot activator was centrifuged (Rotina 420R, Hettich zentrifugen) at 3000 × *g* for 10 min at room temperature (20 °C). The sample was further aliquoted into microfuge tubes, marked, and stored in a biobank at −80 °C until nuclear magnetic resonance (NMR) spectroscopy analysis. In addition, total cholesterol, HDL cholesterol (HDL-C), LDL-C, triglycerides, Apolipoprotein-A1 (Apo-A1), Apolipoprotein-B (Apo-B), Lp(a), creatinine, hemoglobin, and glycated hemoglobin A1c were analyzed in blood samples using standard in-hospital procedures at the Department of Medical Biochemistry, St. Olavs Hospital. In our study, Lp(a) were categorized into elevated Lp(a), defined as Lp(a) > 30 mg/dL, and normal Lp(a), defined as Lp(a) < 30 mg/dL. Information about age (years), body mass index (calculated as kg·m^{-2}), blood pressure, smoking status, diabetes mellitus, medication

use, comorbidities, medically treated hypertension, hyperlipidemia, previous cardiovascular disease (CVD), and heredity for CVD were collected from the hospital medical records at time of inclusion. Medically treated hypertension and hyperlipidemia were defined as patients previously diagnosed with these conditions by a general practitioner or in an outpatient clinic. Previous CVD was defined as patients with previous CAD, stroke, peripheral arterial disease, and/or aortic disease, and heredity of CVD was defined as father or mother with CVD before the age of 55 years and 65 years, respectively.

2.5. Lipoprotein subfraction analysis by nuclear magnetic resonance spectroscopy

NMR spectroscopy was performed using a Bruker Avance III Ultrashield Plus 600 MHz spectrometer (Bruker BioSpin, GmBH, Rheinstetten, Germany) equipped with a 5 mm QCI Cryoprobe at the MR Core Facility, NTNU. Buffer (150 µl, 20 % D_2O with 0.075 M Na_2HPO_4, 6 mM NaN_3, 4.6 mM trimethylsilylpropanoic acid (TSP), pH 7.4) was mixed with 150 µl thawed serum and transferred to 3 mm NMR tubes. Further procedures were fully automated using a SampleJet with Icon-NMR on Topspin 3.1 (Bruker BioSpin). 1D 1H Nuclear Overhauser effect spectroscopy (NOESY) and Carr-Purcell-Meiboom-Gill (CPMG) spectra with water presaturation was obtained at 310 K. The spectra were Fourier transformed to 128 K after 0.3 Hz exponential line broadening.

An automated Bruker IVDr Lipoprotein Subclass Analysis (B.I. LISA^{TM}) was used to quantify 114 lipid variables, where 106 of these variables were considered as lipoprotein subfractions [28] (**Appendix 1**). In total serum, the concentration of cholesterol, triglycerides, Apo-A1, Apolipoprotein-A2 (Apo-A2), and Apo-B/particle number were measured. The ratios LDL-C/HDL-C and Apo-B/Apo-A1 were further calculated. Also, concentrations of cholesterol, free cholesterol, phospholipids, and triglycerides were measured in LDL, HDL, intermediate-density lipoprotein (IDL) and very-low-density lipoprotein (VLDL)), and in their 15 size-based subfractions (LDL 1–6, VLDL 1–5 and HDL 1–4). In addition, Apo-B/particle number was measured in LDL, IDL, VLDL, and LDL 1–6. Apo-A1 and Apo-A2 were measured in HDL and HDL 1–4. With increasing number from 1 to 6 in LDL, 1 to 5 in VLDL, and 1 to 4 in HDL, the particle size decreases. The density ranges of lipoproteins and lipoprotein subfractions are included in **Appendix 2**, and the median with 25- and 75 percentiles for each NMR-derived lipid variable in the study population are included in **Appendix 1**.

2.6. Statistical analyses

The data was analyzed by IBM SPSS Statistics (version 27.0, Armonk, NY: IBM Corp) and R (version 4.0.2). All continuous data are presented as median and inter quartile range and categorical data as frequencies with percentages unless stated otherwise. Lipid variables were assessed for normality by the Shapiro-Wilk test and visual inspection of normal QQ plots. As appropriate, Pearson or Spearman correlations were used to evaluate the correlation between each lipid variable and maxLCBI$_{4mm}$. To estimate the effective number of independent tests for multiple testing correction, principal component analysis was used. Nine principal components explained $>$ 95 % of the variance, and the corrected threshold for assessing statistical significance was therefore $0.05/9 = 0.005$ ($p < .005$). The rationale for this method has been described previously and applied in several metabolic profile studies [29,30].

To study the joint association between lipid variables and maxLCBI$_{4mm}$, the least absolute shrinkage and selection operator (lasso) method for penalized linear regression, as implemented in the *glmnet* package [31] in R, was used. In a model with many predictors, penalized regression aims to reduce the complexity of the model by imposing a penalty, so that the regression coefficients for variables with low predictive value are shrunken towards zero. The method can simultaneously perform parameter estimation and variable selection, as some variables are shrunken to exactly zero. The degree of shrinkage was determined by ten times 10-fold cross-validation, minimizing the mean square error. The uncertainty of the estimated coefficients from the lasso was assessed by bootstrapping. The fitting procedure was repeated for 1000 bootstrap samples, and the uncertainty for each variable was

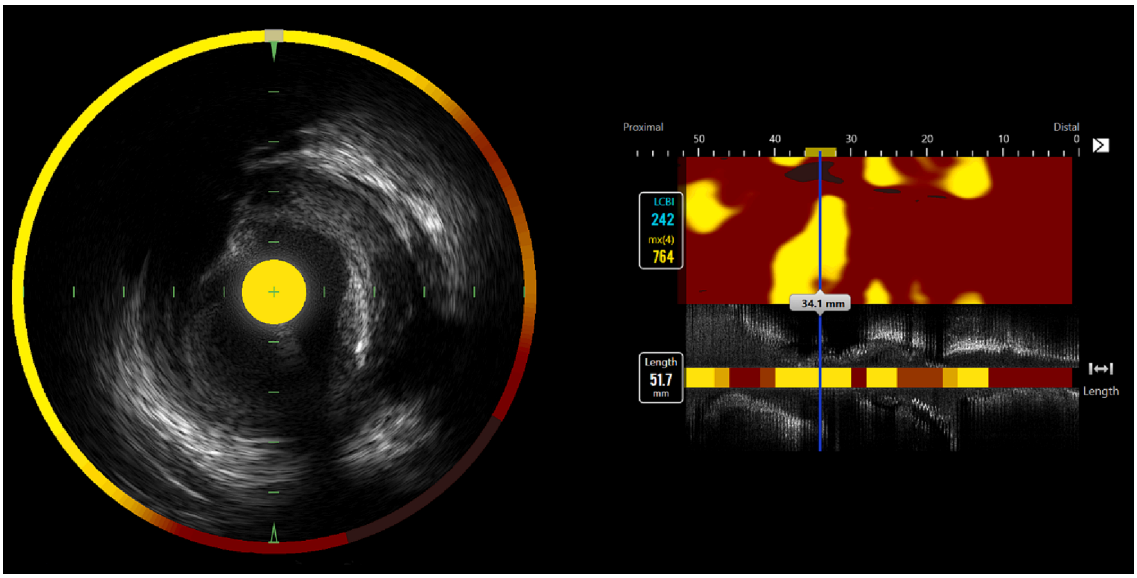

Fig. 1. Left anterior descending artery imaged with combined near-infrared spectroscopy and intravascular ultrasound (NIRS-IVUS) catheter. To the left, a cross-sectional image with a color-coded circumflex that illustrates lipid accumulation within the plaque. Yellow represents high probability of lipids and red represents no lipid. The NIRS-derived chemogram to the right illustrates the maximum lipid core burden index within any 4 mm segment across the entire lesion of 764 (76.4 %).

represented by the proportion of the bootstrap samples for which its coefficient was not set to zero in the estimated model. The model was fitted to two sets of predictors: one model with lipoprotein subfractions, including Lp(a) (N = 107), and one model including both lipoprotein subfractions and 14 established risk factors for CVD (N = 121). The CVD risk factors includes total cholesterol, total triglycerides, LDL-C, HDL-C, LDL-C/HDL-C, Apo-B/Apo-A1, age, body mass index, smoking, diabetes mellitus, medically treated hypertension, hyperlipidemia, previous CVD, and heredity of CVD. All variables were continuous, except from Lp(a) (above/beneath 30 mg/dL), smoking (yes/no), diabetes (yes/no), medically treated hypertension (yes/no), hyperlipidemia (yes/no), previous CVD (yes/no), and heredity of CVD (yes/no).

In the main results, the lipoprotein subfractions and risk factors for CVD that were included in > 50 % of the bootstrap samples and had a non-zero regression coefficient were included in figures and tables. Additional results are presented in appendixes.

3. Results

Out of the 60 patients enrolled in the CENIT-study, this post-hoc analysis included 56 eligible patients with both evaluable NMR spectroscopy data and NIRS-derived maxLCBI$_{4mm}$ data (Appendix 3). Patient characteristics and NIRS-IVUS derived plaque characteristics for the targeted segments are presented in Table 1. NMR measurements of Apo-1, Apo-B, triglycerides, HDL-C, LDL-C, and total cholesterol were compared with gold-standard laboratory measurements to assess the internal validity of NMR spectroscopy. The internal validity was found to be high (Appendix 4).

3.1. *MaxLCBI$_{4mm}$ and lipoprotein subfractions*

The Spearman correlations between maxLCBI$_{4mm}$ and each of the lipid variables were in the range from −0.293 to 0.196, and none were statistically significant after adjustment for multiple testing, with p-values from 0.028 to 0.991 (Appendix 5). Cholesterol in the smallest VLDL subfractions, VLDL-5, was the lipoprotein subfraction most strongly correlated with maxLCBI$_{4mm}$ (corr. coeff = −0.293, p = .028).

In the multivariable analysis, Lp(a) and free cholesterol in the smallest HDL subfractions, HDL-4, were the lipoproteins most strongly associated with maxLCBI$_{4mm}$ according to the percentage presence in the resampled datasets that included each lipoprotein subfraction (Fig. 2). Lp(a) was included in 78.1 % of the resampled dataset, and patients with elevated Lp(a) (n = 23) had an estimated average of 57.0 unit higher maxLCBI$_{4mm}$ values than patients with normal Lp(a) levels (n = 33, Table 2). Free cholesterol in HDL-4 was included in 74.3 % of the resampled dataset, and the maxLCBI$_{4mm}$ increased with an estimated average of 36.5 units for every unit increase (mg/dL) of free cholesterol in HDL-4 (Table 2). Extended results, including all analyzed lipoproteins are listed in Appendix 6.

3.2. *MaxLCBI$_{4mm}$, lipoprotein subfractions, and CVD risk factors*

When including established CVD risk factors in the regression model, the association between maxLCBI$_{4mm}$ and both Lp(a) and free cholesterol in HDL-4 was weakened. Lp(a) and free cholesterol in HDL-4 were included in 67.1 % and 44.6 % of the resampled datasets by bootstrapping, respectively, and had an estimated regression coefficient of zero (Appendix 7).

Among the CVD risk factors, medically treated hypertension was included in 91.7 % of the resampled datasets and had a regression coefficient of −49.5 unit, meaning that patients that were medically treated for hypertension had on average 49.5 units lower maxLCBI$_{4mm}$ compared to patients not medically treated for hypertension (Appendix 7). None of the traditional lipid measurements were found to be potential predictors of maxLCBI$_{4mm}$ as they had a regression coefficient of zero and were included in less than 11 % of the resampled datasets (Table 3).

Table 1
Patient characteristics and NIRS-IVUS derived plaque characteristics for the study population (N = 56).

Variables	Total, N = 56
General	
Age, years	57.5 (52.0–65.0)
Males, (n, %)	53 (94.6 %)
Body mass index, kg·m^{-2}	28.0 (26.1–31.0)
Smoking, current and ex-smoker (n, %)	34 (60.7 %)
Systolic blood pressure, mmHg	143.0 (133.2–151.0)
Diastolic blood pressure, mmHg	84.5 (80.0–89.0)
Medical history	
Diabetes mellitus (n, %)	7 (12 %)
Hypertension (n, %)	30 (54 %)
Hyperlipidemia (n, %)	19 (34 %)
Heredity of premature cardiovascular disease (n, %)	47 (84 %)
Prior history of cardiovascular disease (n, %)	27 (48 %)
Medication	
Statins (n, %)	56 (100 %)
Dual antiplatelet therapy (n, %)	56 (100 %)
Combined therapy with Ezetimibe (n, %)	2 (4 %)
Diuretics (n, %)	6 (10.7 %)
Calcium blockers (n, %)	11 (19.6 %)
Betablockers (n, %)	20 (36 %)
ACE inhibitors/angiotensin II receptor antagonists (n, %)	29 (52 %)
Clinical measurements	
Total cholesterol, mmol/L	3.5 (3.2–4.1)
Low-density lipoprotein cholesterol, mmol/L	2.0 (1.7–2.5)
High-density lipoprotein, mmol/L	1.0 (0.9–1.1)
Triglycerides, mmol/L	1.3 (1.0–1.7)
Apolipoprotein A1, g/L	1.3 (1.2–1.4)
Apolipoprotein B, g/L	0.7 (0.6–0.8)
Lipoprotein a, mg/L	147.0 (100–811)
Creatinine, μmol/L	79.0 (72.0–88.5)
Hemoglobin, g/dL	14.8 (14.0–15.4)
Glycosylated hemoglobin, %	5.5 (5.2–5.9)
Target segments	
Left anterior descending artery (n, %)	24 (43 %)
Circumflex artery (n, %)	13 (23 %)
Right coronary artery (n, %)	19 (34 %)
NIRS-IVUS derived plaque characteristics for the targeted segments	
maxLCBI$_{4mm}$	326.0 (172.5–402.5)
Total lipid core burden index at region of interest	111.5 (39.0–182.5)
Plaque burden, %	49.2 (42.6–54.8)
Minimal lumen area, mm^2	4.8 (3.9–6.6)
Stenosis at minimal lumen area, %	64.2 (55.1–73.6)
Plaque volume, mm^3	155.5 (93.5–261.7)
Vessel volume, mm^3	292.3 (191.0–469.6)
Lumen volume, mm^3	154.2 (102.1–231.4)
Segment length, mm	20.5 (14.3–29.2)

Data are presented as median with 25 and 75 percentiles or numbers with percentages. NIRS-IVUS, near-infrared spectroscopy intravascular ultrasound; ACE inhibitors, Angiotensin-converting-enzyme inhibitors; maxLBI$_{4mm}$, the maximum lipid core burden index within any 4 mm segment across the entire lesion; NIRS-IVUS, near-infrared spectroscopy intravascular ultrasound.

4. Discussion

In this study, we investigated the association between circulating lipoprotein subfractions and lipid content in coronary atheromatous plaques in statin-treated patients with stable CAD undergoing PCI. The main findings were: 1) Lp(a) and free cholesterol in the smallest HDL subfractions, HDL-4, were the lipoprotein subfractions with the strongest potential as predictors of coronary lipid content measured as maxLCBI$_{4mm}$, 2) after including established CVD risk factors in the regression model, the association between coronary lipid content and both Lp(a) and free cholesterol in HDL-4 was weakened, and 3) we did not detect any associations between traditional lipid measurements and coronary lipid content.

To the best of our knowledge, this is the first study to investigate the association between a large number of lipoprotein subfractions and lipid

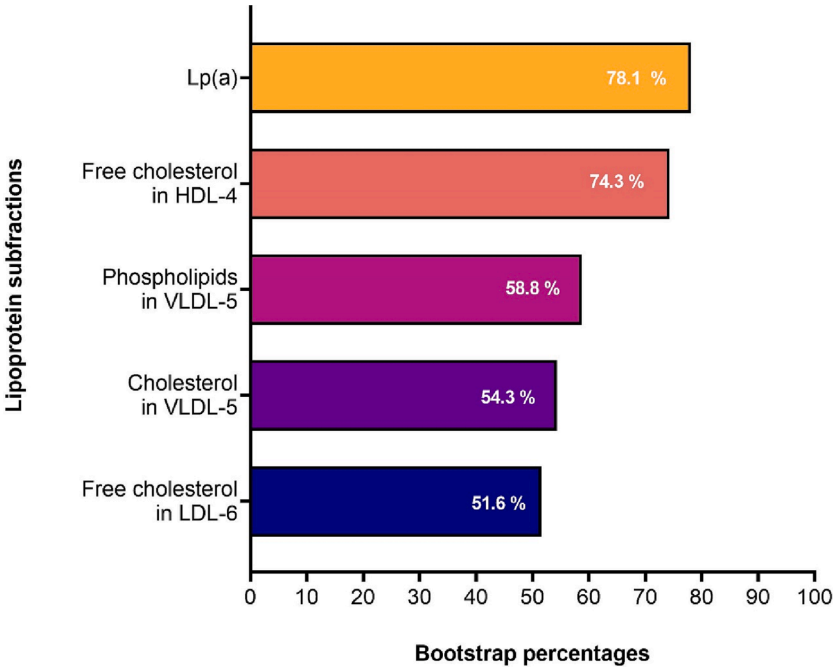

Fig. 2. Lipoprotein subfractions that had the strongest potential as predictors for maxLCBI$_{4mm}$ according to the percentage presence in the resampled datasets by bootstrapping. The presented lipoprotein subfractions were present in >50 % of the resampled dataset and had a non-zero regression coefficient from lasso. Lp(a), lipoprotein a; HDL-4, high-density lipoprotein 4; VLDL-5; very-low-density lipoprotein 5; LDL-6, low-density lipoprotein 6; Lasso, least absolute shrinkage, and selection operator.

Table 2
The estimated lasso regression coefficients of the lipoprotein subfractions that were present in > 50 % of the resampled datasets by bootstrapping.

Lipoprotein subfractions	Regression coefficient
Lp(a)	57.0
Free cholesterol in HDL-4	36.5
Phospholipids in VLDL-5	−78.5
Cholesterol in VLDL-5	−32.5
Free cholesterol in LDL-6	21.9

Lasso, least absolute shrinkage, and selection operator; Lp(a), lipoprotein a; HDL-4, high-density lipoprotein 4; VLDL-5, very-low-density lipoprotein 5; LDL-6, low-density lipoprotein 6.

Table 3
The percentage presence of the traditional lipid measurements in the resampled datasets by bootstrapping.

Clinical biomarkers	Percentage included
LDL-C/HDL-C	10.2
HDL-C (mg/dL)	3.7
Total cholesterol (mg/dL)	2.5
Apo-B/Apo-A1	2.1
LDL-C (mg/dL)	1.3
Total triglycerides (mg/dL)	0

Percent included, how frequently the traditional lipid measurements were included in the model across the 1000 bootstrap samples; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Apo-B/Apo-A1, apolipoprotein B/apolipoprotein A1.

content in coronary atheromatous plaques measured as maxLCBI$_{4mm}$ by NIRS. Although hyperlipidemia, and particularly high levels of LDL-C, is considered a major risk factor for CVD, only a few studies have assessed the correlation or association between coronary lipid content measured as maxLCBI$_{4mm}$ and traditional lipid measurements in patients with CVD [21–24]. One study demonstrated that the percent change in HDL-C was negatively associated with the percent change in maxLCBI$_{4mm}$ after 13 months follow-up in patients with acute coronary syndrome or stable CAD [22]. Another study found a negative correlation between HDL-C and maxLCBI$_{4mm}$ in patients with acute coronary syndrome or stable CAD with a maxLCBI$_{4mm} \geq 323$, but not in patients with a maxLCBI$_{4mm} < 323$ [21]. In these two studies, 78 % and 85.7 % of the patients were on statin-therapy, respectively. In statin-treated patients with CAD, an association between LDL-C and maxLCBI$_{4mm}$ was recently demonstrated, while other traditional lipid measurements were not included in this analysis [23]. Furthermore, a post-hoc analysis of the Lipid Rich Plaque Study that included 984 patients, investigated the potential correlation between maxLCBI$_{4mm}$ and both LDL- and HDL-C, and did not detect any significant correlations among statin-naïve patients, statin-treated patients, or in the total population [24]. This is in line with the present study, as none of the traditional lipid measurements were associated with lipid content in coronary atheromatous plaques. This may be due to the effect of lipid-lowering therapy as all patients were on stable statin-treatment for at least 6 weeks prior to inclusion. Statins are known to reduce LDL, VLDL and IDL cholesterol, and slightly increase HDL-C and Lp(a), and to provide positive effects on coronary lipid content and plaque stabilization [26]. Lp(a) and free cholesterol in HDL-4 were the lipoprotein subfractions most strongly associated with coronary lipid content in our study, suggesting that these lipoprotein subfractions may not be substantially affected by statins and may provide additional information with respect to coronary plaque composition compared to traditional lipid measurements.

Genetic and observational evidence has convincingly demonstrated a causal and linear relationship between high concentrations of Lp(a) and atherosclerotic CVD and cardiovascular- and all-cause mortality [20,32–34]) . Lp(a) levels are slightly increased by statins, but several *meta*-analyses support that the statin-induced changes in Lp(a) levels are not clinically significant [35–37]. Thus, statins are not considered to change the Lp(a)-associated risk of CVD. In our study, Lp(a) were categorized into elevated Lp(a), defined as Lp(a) > 30 mg/dL, and

normal Lp(a), defined as Lp(a) < 30 mg/dL, which was the most used approach at the time of inclusion [34]. We found that Lp(a) was the lipoprotein most strongly associated with $maxLCBI_{4mm}$, suggesting that high levels of Lp(a) may predict coronary lipid content in patients with stable CAD. A study by Nakamura et al. [23] recently demonstrated that Lp(a) was associated with $maxLCBI_{4mm}$ in patients with both CAD and diabetes, while not in patients with CAD and no diabetes. Lp(a) is considered an important promoter of plaque vulnerability, partly by binding to oxidative phospholipids with pro-inflammatory properties and housing the glycation of Apo-B [38], mechanisms which are known to be increased in diabetic patients. Another study using optical coherence tomography found that patients with Lp(a) > 30 mg/dL had more high-risk plaques, which included more lipid-rich plaques and thinner cap fibroatheromas, and wider lipid arcs, compared to patients with Lp (a) < 30 mg/dL [39].

HDL is generally known to be negatively associated with coronary atherosclerosis, but a causal association between HDL-C and CVD has been challenged by large Mendelian randomization studies and HDL-C raising drug trials [40,41]. Since HDL is highly heterogeneous, it is suggested that not all HDL subfractions holds atheroprotective properties and that total HDL-C is not a sufficient measure of its protective properties. To the best of our knowledge, no previous study has investigated the association between lipid content in coronary artery plaque, measured as $maxLCBI_{4mm}$ by NIRS, and HDL subfractions in patients with CAD, nor the association between HDL subfractions and cardiovascular outcomes. Even though we did not detect an association between lipid content in coronary plaque and serum HDL-C, we found free cholesterol in the smallest HDL subfractions, HDL-4, to be one of the lipoprotein subfractions most strongly associated with $maxLCBI_{4mm}$. The evidence of free cholesterol in HDL-4 as a potential predictor of lipid content was however substantially reduced after including established risk factors in the regression model. Although the role of particle size remains controversial, our study supports the assumptions that not all HDL subfractions necessarily have atheroprotective properties, that the smallest subfractions may increase the CVD risk, and that HDL subfractions may add information beyond total HDL-C. Since statins only induce a small increase in HDL-C, HDL subfractions may not be substantially affected, and could therefore represent valuable markers of coronary lipid content in statin-treated patients with stable CAD.

In the regression analysis that included CVD risk factors and lipoprotein subfractions, patients with medically treated hypertension had on average 49.5 units lower $maxLCBI_{4mm}$ compared to patients without known hypertension. In our study, hypertension that required medical treatment was diagnosed prior to inclusion. Accordingly, these patients may have been offered more intensive preventive therapy with influence on plaque lipid content.

There are some limitations to address. First, our sample size was limited. Our study included 53 males and 3 females, and since the lipoprotein subfraction profile may be sex-specific [42,43], our results may only be applicable for males. Secondly, PCI was a requirement for inclusion, and for ethical reasons, blood samples were taken after PCI when patients were found eligible. The vessel trauma induced by PCI may have affected the lipoprotein subfraction profile. In addition, all patients had to be on stable statin treatment for at least 6 weeks prior to inclusion, which influence the lipoprotein subfraction profile. However, for lipoprotein subfractions to be clinically useful as a biomarker for CVD risk, it should also be applicable to patients on statins. A strength in our study is that multivessel imaging was conducted to ensure detection of the most diseased vessel. $MaxLCBI_{4mm}$ was used to target the most diseased coronary segment, but this is not necessarily representative for the total atherosclerotic burden. Another strength is that all NIRS-IVUS data were analyzed at an independent core facility.

5. Conclusion

In this study of lipoprotein subfractions and coronary lipid content, Lp(a) and free cholesterol in the smallest HDL subfractions, HDL-4, had the highest potential as predictors of coronary lipid content in statin-treated patients with stable CAD. However, only moderate evidence was demonstrated, and adjusting for established risk factors for CVD weakened the associations. Further and larger studies are needed to assess the potential of circulating lipoprotein subfractions as meaningful markers both for lipid content in coronary atheromatous plaques and as CVD risk markers.

6. Sources of funding

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

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Appendix 1. .

Lipid variables (N = 114) with its matrix, analyte, and unit analyzed by nuclear magnetic resonance spectroscopy, and the calculated median and percentiles for each lipid variable in our study population (N = 56).

Lipid variables	Unit	Median (25–75 percentiles)
Total Plasma Triglycerides*	mg/dL	118.6 (88.5–162.4)
Total Plasma Cholesterol*	mg/dL	137.6 (122.7–165.5)
LDL Cholesterol*	mg/dL	68.4 (56.9–84.6)
HDL Cholesterol*	mg/dL	40.7 (36.4–45.0)
Total Plasma Apo-A1	mg/dL	111.9 (105.3–119.4)
Total Plasma Apo-A2	mg/dL	22.5 (20.6–24.8)
Total Plasma Particle Number	nmol/L	1140.6 (1013.9–1414.7)
Total Plasma Apo-B	mg/dL	62.7 (55.8–77.8)
LDL cholesterol/HDL cholesterol*	–	1.6 (1.4–2.1)
Apo-B/Apo-A1*	–	0.6 (0.5–0.72)

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Lipid variables	Unit	Median (25–75 percentiles)
VLDL Particle Number	nmol/L	153.8 (124.7–210.4)
IDL Particle Number	nmol/L	46.3 (25.6–75.4)
LDL Particle Number	nmol/L	913.9 (745.5–1117.4)
LDL-1 Particle Number	nmol/L	144.3 (128.1–179.7)
LDL-2 Particle Number	nmol/L	102.4 (77.7–128.2)
LDL-3 Particle Number	nmol/L	111.1 (74.5–147.5)
LDL-4 Particle Number	nmol/L	107.4 (58.1–158.2)
LDL-5 Particle Number	nmol/L	131.1 (93.1–195.6)
LDL-6 Particle Number	nmol/L	278.4 (236.1–367.2)
VLDL Triglycerides	mg/dL	86.0 (60.4–117.3)
IDL Triglycerides	mg/dL	10.1 (4.8–17.8)
LDL Triglycerides	mg/dL	13.2 (10.3–16.3)
HDL Triglycerides	mg/dL	8.5 (7.4–9.9)
VLDL Cholesterol	mg/dL	15.4 (11.2–23.7)
IDL Cholesterol	mg/dL	4.5 (1.2–10.3)
LDL Cholesterol†	mg/dL	68.4 (56.9–84.6)
HDL Cholesterol†	mg/dL	40.7 (36.4–45.0)
VLDL Free Cholesterol	mg/dL	9.2 (7.3–12.8)
IDL Free Cholesterol	mg/dL	1.0 (0.2–2.8)
LDL Free Cholesterol	mg/dL	23.9 (20.7–28.9)
HDL Free Cholesterol	mg/dL	11.9 (10.4–13.7)
VLDL Phospholipids	mg/dL	21.5 (16.4–29.5)
IDL Phospholipids	mg/dL	2.9 (1.4–6.3)
LDL Phospholipids	mg/dL	41.8 (35.1–50.3)
HDL Phospholipids	mg/dL	53.8 (49.4–60.3)
HDL Apo-A1	mg/dL	109.9 (103.2–118.4)
HDL Apo-A2	mg/dL	23.4 (21.4–25.9)
VLDL Apo-B	mg/dL	8.5 (6.9–11.6)
IDL Apo-B	mg/dL	2.5 (1.4–4.1)
LDL Apo-B	mg/dL	50.3 (41.0–61.4)
VLDL-1 Triglycerides	mg/dL	47.2 (33.2–66.9)
VLDL-2 Triglycerides	mg/dL	14.6 (10.3–20.2)
VLDL-3 Triglycerides	mg/dL	11.0 (8.1–16.2)
VLDL-4 Triglycerides	mg/dL	8.3 (7.3–11.3)
VLDL-5 Triglycerides	mg/dL	3.6 (3.3–4.1)
VLDL-1 Cholesterol	mg/dL	5.7 (3.8–9.1)
VLDL-2 Cholesterol	mg/dL	1.9 (1.1–3.1)
VLDL-3 Cholesterol	mg/dL	1.9 (1.0–3.6)
VLDL-4 Cholesterol	mg/dL	3.2 (2.3–5.3)
VLDL-5 Cholesterol	mg/dL	1.6 (1.4–1.9)
VLDL-1 Free Cholesterol	mg/dL	2.2 (1.2–3.7)
VLDL-2 Free Cholesterol	mg/dL	1.0 (0.6–1.5)
VLDL-3 Free Cholesterol	mg/dL	1.1 (0.7–2.0)
VLDL-4 Free Cholesterol	mg/dL	1.2 (0.8–2.2)
VLDL-5 Free Cholesterol	mg/dL	0.7 (0.5–0.9)
VLDL-1 Phospholipids	mg/dL	6.9 (4.1–10.0)
VLDL-2 Phospholipids	mg/dL	3.2 (2.0–4.4)
VLDL-3 Phospholipids	mg/dL	3.1 (2.1–4.9)
VLDL-4 Phospholipids	mg/dL	3.6 (3.0–5.2)
VLDL-5 Phospholipids	mg/dL	2.3 (2.0–2.5)
LDL-1 Triglycerides	mg/dL	4.5 (3.7–5.7)
LDL-2 Triglycerides	mg/dL	1.3 (0.9–1.7)
LDL-3 Triglycerides	mg/dL	2.1 (1.8–2.4)
LDL-4 Triglycerides	mg/dL	1.2 (0.8–1.8)
LDL-5 Triglycerides	mg/dL	1.4 (0.9–1.9)
LDL-6 Triglycerides	mg/dL	4.4 (3.7–5.8)
LDL-1 Cholesterol	mg/dL	12.5 (10.1–16.1)
LDL-2 Cholesterol	mg/dL	8.4 (5.9–11.3)
LDL-3 Cholesterol	mg/dL	9.0 (5.5–13.1)
LDL-4 Cholesterol	mg/dL	9.5 (5.7–13.5)
LDL-5 Cholesterol	mg/dL	10.1 (7.4–14.7)
LDL-6 Cholesterol	mg/dL	19.3 (16.1–25.4)
LDL-1 Free Cholesterol	mg/dL	3.8 (3.1–4.7)
LDL-2 Free Cholesterol	mg/dL	2.9 (2.1–3.8)
LDL-3 Free Cholesterol	mg/dL	3.5 (2.5–4.5)
LDL-4 Free Cholesterol	mg/dL	3.3 (2.6–4.4)
LDL-5 Free Cholesterol	mg/dL	3.2 (2.6–4.1)
LDL-6 Free Cholesterol	mg/dL	4.9 (4.2–6.1)
LDL-1 Phospholipids	mg/dL	7.9 (6.9–9.8)
LDL-2 Phospholipids	mg/dL	5.3 (3.9–6.8)
LDL-3 Phospholipids	mg/dL	5.6 (3.6–7.6)
LDL-4 Phospholipids	mg/dL	5.8 (3.9–7.9)
LDL-5 Phospholipids	mg/dL	5.8 (4.5–8.0)
LDL-6 Phospholipids	mg/dL	10.5 (8.8–13.7)
LDL-1 Apo-B	mg/dL	7.9 (7.0–9.9)
LDL-2 Apo-B	mg/dL	5.6 (4.3–7.1)

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Lipid variables	Unit	Median (25–75 percentiles)
LDL-3 Apo-B	mg/dL	6.1 (4.1–8.1)
LDL-4 Apo-B	mg/dL	5.9 (3.2–8.7)
LDL-5 Apo-B	mg/dL	7.2 (5.1–10.8)
LDL-6 Apo-B	mg/dL	15.3 (13.0–20.2)
HDL-1 Triglycerides	mg/dL	2.0 (1.6–2.7)
HDL-2 Triglycerides	mg/dL	1.2 (1.0–1.5)
HDL-3 Triglycerides	mg/dL	1.6 (1.4–1.9)
HDL-4 Triglycerides	mg/dL	3.4 (2.9–4.0)
HDL-1 Cholesterol	mg/dL	9.9 (7.8–13.0)
HDL-2 Cholesterol	mg/dL	4.6 (3.3–5.4)
HDL-3 Cholesterol	mg/dL	6.8 (6.0–7.5)
HDL-4 Cholesterol	mg/dL	19.2 (17.3–20.7)
HDL-1 Free Cholesterol	mg/dL	2.7 (1.9–3.2)
HDL-2 Free Cholesterol	mg/dL	1.1 (0.8–1.5)
HDL-3 Free Cholesterol	mg/dL	1.8 (1.4–2.1)
HDL-4 Free Cholesterol	mg/dL	4.0 (3.5–4.5)
HDL-1 Phospholipids	mg/dL	10.4 (8.2–13.6)
HDL-2 Phospholipids	mg/dL	7.5 (5.4–8.6)
HDL-3 Phospholipids	mg/dL	11.1 (9.5–12.4)
HDL-4 Phospholipids	mg/dL	25.4 (23.4–27.8)
HDL-1 Apo-A1	mg/dL	8.5 (6.2–13.5)
HDL-2 Apo-A1	mg/dL	11.6 (10.3–13.4)
HDL-3 Apo-A1	mg/dL	19.9 (17.8–22.0)
HDL-4 Apo-A1	mg/dL	70.7 (66.5–77.3)
HDL-1 Apo-A2	mg/dL	0.5 (0.1–1.0)
HDL-2 Apo-A2	mg/dL	1.3 (0.8–1.8)
HDL-3 Apo-A2	mg/dL	3.8 (3.3–4.2)
HDL-4 Apo-A2	mg/dL	17.4 (15.8–19.3)

LDL, low-density lipoprotein; HDL, high-density lipoprotein; Apo-A1, apolipoprotein A1; Apo-A2, apolipoprotein A2; Apo-B, apolipoprotein B; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein. *Included as a CVD risk factor in the statistical analyses, †Duplicate, removed from all statistical analyses.

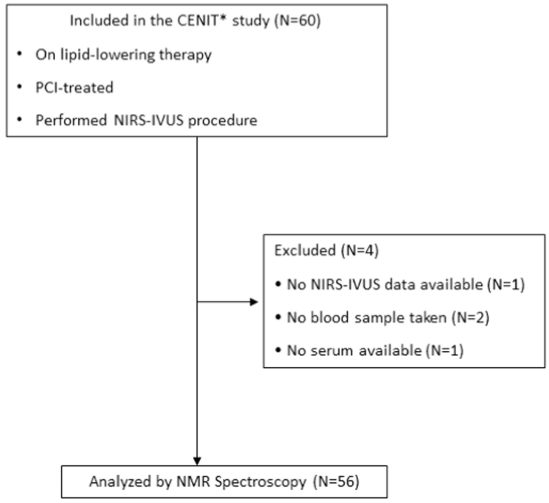
Appendix 2. .

Density ranges of lipoproteins and lipoprotein subfractions.

	Ranges, kg/L
Main lipoprotein fractions	
LDL	1.019–1.063
VLDL*	0.950–1.006
IDL	1.006–1.019
HDL	1.063–1.210
Low-density lipoprotein subfractions	
LDL-1	1.019–1.031
LDL-2	1.031–1.034
LDL-3	1.034–1.037
LDL-4	1.037–1.040
LDL-5	1.040–1.044
LDL-6	1.044–1.063
High-density lipoprotein subfractions	
HDL-1	1.063–1.100
HDL-2	1.100–1.112
HDL-3	1.112–1.125
HDL-4	1.125–1.210

LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein. *The density ranges for VLDL subfractions 1–5 are specified in *Lindgren FT, Jensen LL, Hatch FT (1972). The isolation and quantitative analysis of serum lipoproteins. In Nelson GJ (ed.) Blood lipids and lipoproteins: Quantitation, composition and metabolism. Wiley-Interscience, New York, p 181–274.*

Appendix 3. . Flowchart of the enrollment.



* Vesterbekkmo, E. K., Madssen, E., Aamot Aksetøy, I. L., Follestad, T., Nilsen, H. O., Hegbom, K.,Wisløff, U., & Wiseth, R. (2022). CENIT (Impact of Cardiac Exercise Training on Lipid Content in Coronary Atheromatous Plaques Evaluated by Near-Infrared Spectroscopy): A Randomized Trial. *J Am Heart Assoc*, *11*(10), e024705. https://doi.org/10.1161/jaha.121.024705.

Appendix 4. .

The Spearman correlation between lipid variables from nuclear magnetic resonance (NMR) spectroscopy and lipid measurements from gold-standard laboratory measurements, indicating high internal validity.

Gold-standard laboratory measurements	Unit	NMR spectroscopy measurements	Unit	Spearman correlation coefficient	p-value	Number
Apolipoprotein A1	g/L	Apolipoprotein A1	mg/dL	0.851	<0.001	56
Apolipoprotein B	g/L	Apolipoprotein B	mg/dL	0.921	<0.001	56
Total triglycerides	mmol/L	Total triglycerides	mg/dL	0.944	<0.001	56
HDL-C	mmol/L	HDL-C	mg/dL	0.874	<0.001	56
LDL-C	mmol/L	LDL-C	mg/dL	0.838	<0.001	56
Total cholesterol	mmol/L	Total cholesterol	mg/dL	0.937	<0.001	56

NMR, nuclear magnetic resonance; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Appendix 5. .

Spearman correlations between max$LCBI_{4mm}$ and lipid variables (N = 112).

Lipoprotein variables	Spearman correlation coefficient	p-value	Number
Total Plasma Triglycerides	−0.107	0.431	56
Total Plasma Cholesterol	−0.030	0.825	56
LDL Cholesterol	−0.052	0.702	56
HDL Cholesterol	0.099	0.466	56
Total Plasma Apo-A1	0.069	0.615	56
Total Plasma Apo-A2	0.162	0.234	56
Total Plasma Apo-B	−0.058	0.671	56
LDL cholesterol/HDL cholesterol	−0.071	0.605	56
Apo-B/Apo-A1	−0.062	0.652	56
Total Plasma Particle Number	−0.058	0.671	56
VLDL Particle Number	−0.114	0.404	56
IDL Particle Number	−0.060	0.661	56
LDL Particle Number	−0.035	0.797	56
LDL-1 Particle Number	−0.012	0.927	56
LDL-2 Particle Number	−0.045	0.740	56
LDL-3 Particle Number	0.005	0.972	56
LDL-4 Particle Number	−0.033	0.810	56
LDL-5 Particle Number	−0.009	0.947	56
LDL-6 Particle Number	0.085	0.533	56
VLDL Triglycerides	−0.063	0.646	56

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Lipoprotein variables	Spearman correlation coefficient	p-value	Number
IDL Triglycerides	−0.092	0.501	56
LDL Triglycerides	0.021	0.880	56
HDL Triglycerides	−0.158	0.245	56
VLDL Cholesterol	−0.126	0.355	56
IDL Cholesterol	−0.043	0.755	56
VLDL Free Cholesterol	−0.134	0.323	56
IDL Free Cholesterol	−0.050	0.716	56
LDL Free Cholesterol	−0.028	0.838	56
HDL Free Cholesterol	0.120	0.377	56
VLDL Phospholipids	−0.089	0.514	56
IDL Phospholipids	−0.036	0.791	56
LDL Phospholipids	−0.051	0.707	56
HDL Phospholipids	0.086	0.527	56
HDL Apo-A1	0.077	0.571	56
HDL Apo-A2	0.173	0.202	56
VLDL Apo-B	−0.115	0.398	56
IDL Apo-B	−0.058	0.671	56
LDL Apo-B	−0.035	0.797	56
VLDL-1 Triglycerides	−0.063	0.646	56
VLDL-2 Triglycerides	−0.032	0.817	56
VLDL-3 Triglycerides	−0.045	0.740	56
VLDL-4 Triglycerides	−0.110	0.418	56
VLDL-5 Triglycerides	−0.190	0.161	56
VLDL-1 Cholesterol	−0.095	0.484	56
VLDL-2 Cholesterol	−0.089	0.517	56
VLDL-3 Cholesterol	−0.051	0.709	56
VLDL-4 Cholesterol	−0.125	0.358	56
VLDL-5 Cholesterol	−.293*	0.028	56
VLDL-1 Free Cholesterol	−0.085	0.535	56
VLDL-2 Free Cholesterol	−0.096	0.480	56
VLDL-3 Free Cholesterol	−0.072	0.598	56
VLDL-4 Free Cholesterol	−0.133	0.329	56
VLDL-5 Free Cholesterol	−0.229	0.090	56
VLDL-1 Phospholipids	−0.066	0.629	56
VLDL-2 Phospholipids	−0.027	0.842	56
VLDL-3 Phospholipids	−0.034	0.804	56
VLDL-4 Phospholipids	−0.152	0.265	56
VLDL-5 Phospholipids	−0.263	0.050	56
LDL-1 Triglycerides	−0.072	0.600	56
LDL-2 Triglycerides	−0.067	0.621	56
LDL-3 Triglycerides	−0.138	0.312	56
LDL-4 Triglycerides	−0.057	0.676	56
LDL-5 Triglycerides	−0.029	0.834	56
LDL-6 Triglycerides	0.156	0.250	56
LDL-1 Cholesterol	−0.053	0.699	56
LDL-2 Cholesterol	−0.034	0.803	56
LDL-3 Cholesterol	0.004	0.976	56
LDL-4 Cholesterol	−0.039	0.773	56
LDL-5 Cholesterol	−0.006	0.964	56
LDL-6 Cholesterol	0.108	0.427	56
LDL-1 Free Cholesterol	−0.035	0.797	56
LDL-2 Free Cholesterol	−0.009	0.948	56
LDL-3 Free Cholesterol	0.039	0.777	56
LDL-4 Free Cholesterol	0.008	0.951	56
LDL-5 Free Cholesterol	0.024	0.863	56
LDL-6 Free Cholesterol	0.147	0.281	56
LDL-1 Phospholipids	−0.064	0.637	56
LDL-2 Phospholipids	−0.029	0.832	56
LDL-3 Phospholipids	0.003	0.980	56
LDL-4 Phospholipids	−0.042	0.761	56
LDL-5 Phospholipids	−0.013	0.927	56
LDL-6 Phospholipids	0.102	0.454	56
LDL-1 Apo-B	−0.015	0.913	56
LDL-2 Apo-B	−0.043	0.751	56
LDL-3 Apo-B	0.005	0.972	56
LDL-4 Apo-B	−0.033	0.809	56
LDL-5 Apo-B	−0.009	0.947	56
LDL-6 Apo-B	0.085	0.533	56
HDL-1 Triglycerides	−0.158	0.245	56
HDL-2 Triglycerides	−0.173	0.202	56
HDL-3 Triglycerides	−0.111	0.417	56
HDL-4 Triglycerides	−0.153	0.261	56
HDL-1 Cholesterol	0.076	0.580	56
HDL-2 Cholesterol	−0.002	0.991	56
HDL-3 Cholesterol	0.160	0.239	56
HDL-4 Cholesterol	0.127	0.351	56

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Lipoprotein variables	Spearman correlation coefficient	p-value	Number
HDL-1 Free Cholesterol	0.100	0.464	56
HDL-2 Free Cholesterol	0.161	0.235	56
HDL-3 Free Cholesterol	0.150	0.271	56
HDL-4 Free Cholesterol	0.196	0.147	56
HDL-1 Phospholipids	0.050	0.712	56
HDL-2 Phospholipids	0.008	0.956	56
HDL-3 Phospholipids	0.159	0.241	56
HDL-4 Phospholipids	0.122	0.372	56
HDL-1 Apo-A1	−0.055	0.688	56
HDL-2 Apo-A1	0.011	0.935	56
HDL-3 Apo-A1	0.099	0.470	56
HDL-4 Apo-A1	0.106	0.436	56
HDL-1 Apo-A2	0.045	0.742	56
HDL-2 Apo-A2	0.095	0.487	56
HDL-3 Apo-A2	0.161	0.235	56
HDL-4 Apo-A2	0.098	0.472	56

LDL, low-density lipoprotein; HDL, high-density lipoprotein; Apo-A1, apolipoprotein A1; Apo-A2, apolipoprotein A2; Apo-B, apolipoprotein B; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein.

Appendix 6. .

The estimated regression coefficient from the least absolute shrinkage and selection operator method with percentage of models from the 1000 bootstrap samples that include each of the lipoprotein subfractions.

Lipoprotein subfractions	Estimated regression coefficient	Percent included
Lipoprotein a	56.96	78.1
Total Plasma Apo-A1	0	18.6
Total Plasma Apo-A2	0	5.9
Total Plasma Apo-B	0	0.8
VLDL Particle Number	0	4.5
IDL Particle Number	0	3.7
LDL Particle Number	0	0.2
LDL-1 Particle Number	0	1.2
LDL-2 Particle Number	0	11.7
LDL-3 Particle Number	0	4
LDL-4 Particle Number	0	2.5
LDL-5 Particle Number	0	0.8
LDL-6 Particle Number	0	14.4
VLDL Triglycerides	0	0.3
IDL Triglycerides	0	5.4
LDL Triglycerides	0	12.8
HDL Triglycerides	−3.132	44.3
VLDL Cholesterol	0	0.8
IDL Cholesterol	0	4.7
VLDL Free Cholesterol	0	2.1
IDL Free Cholesterol	0	5.1
LDL Free Cholesterol	0	1.5
HDL Free Cholesterol	0	27.3
VLDL Phospholipids	0	0.2
IDL Phospholipids	0	1.3
LDL Phospholipids	0	0.9
HDL Phospholipids	0	2.5
HDL Apo-A1	0	6.2
HDL Apo-A2	0	6.9
VLDL Apo-B	0	3.4
IDL Apo-B	0	1.7
LDL Apo-B	0	0.1
VLDL-1 Triglycerides	0	7.9
VLDL-2 Triglycerides	0	2.8
VLDL-3 Triglycerides	0	2.3
VLDL-4 Triglycerides	0	5.2
VLDL-5 Triglycerides	0	31.3
VLDL-1 Cholesterol	0	3.5
VLDL-2 Cholesterol	0	4.9
VLDL-3 Cholesterol	0	7
VLDL-4 Cholesterol	0	9.6
VLDL-5 Cholesterol	–32.512	54.3
VLDL-1 Free Cholesterol	0	3.1
VLDL-2 Free Cholesterol	0	5.2
VLDL-3 Free Cholesterol	0	0.4
VLDL-4 Free Cholesterol	0	1.6
VLDL-5 Free Cholesterol	0	23.6
VLDL-1 Phospholipids	0	2.3

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Lipoprotein subfractions	Estimated regression coefficient	Percent included
VLDL-2 Phospholipids	0	1
VLDL-3 Phospholipids	0	4.6
VLDL-4 Phospholipids	0	4.1
VLDL-5 Phospholipids	−78.469	58.8
LDL-1 Triglycerides	0	21.9
LDL-2 Triglycerides	0	22.4
LDL-3 Triglycerides	0	12.4
LDL-4 Triglycerides	0	18.9
LDL-5 Triglycerides	0	8.4
LDL-6 Triglycerides	0	27.7
LDL-1 Cholesterol	0	24.3
LDL-2 Cholesterol	0	11
LDL-3 Cholesterol	0	15.8
LDL-4 Cholesterol	0	2.9
LDL-5 Cholesterol	0	4.8
LDL-6 Cholesterol	0	3.2
LDL-1 Free Cholesterol	0	7.4
LDL-2 Free Cholesterol	0	15.2
LDL-3 Free Cholesterol	0	4.8
LDL-4 Free Cholesterol	0	4.1
LDL-5 Free Cholesterol	0	3.8
LDL-6 Free Cholesterol	21.949	51.6
LDL-1 Phospholipids	0	3.2
LDL-2 Phospholipids	−3.317	18.8
LDL-3 Phospholipids	−2.553	21.2
LDL-4 Phospholipids	−6.362	26.5
LDL-5 Phospholipids	0	5.2
LDL-6 Phospholipids	0	0.7
LDL-1 Apo-B	0	5
LDL-2 Apo-B	0	9.9
LDL-3 Apo-B	0	4
LDL-4 Apo-B	0	2.4
LDL-5 Apo-B	0	0.7
LDL-6 Apo-B	0	13.2
HDL-1 Triglycerides	−26.616	31.5
HDL-2 Triglycerides	0	11.4
HDL-3 Triglycerides	0	7.5
HDL-4 Triglycerides	0	31.7
HDL-1 Cholesterol	0	15.4
HDL-2 Cholesterol	0	1.4
HDL-3 Cholesterol	18.396	39.5
HDL-4 Cholesterol	0	11.6
HDL-1 Free Cholesterol	0	5.7
HDL-2 Free Cholesterol	15.901	33.8
HDL-3 Free Cholesterol	0	14.9
HDL-4 Free Cholesterol	36.547	74.3
HDL-1 Phospholipids	0	5.9
HDL-2 Phospholipids	0	3.2
HDL-3 Phospholipids	0	13.9
HDL-4 Phospholipids	0	9
HDL-1 Apo-A1	0	13.2
HDL-2 Apo-A1	0	17.4
HDL-3 Apo-A1	0	7.6
HDL-4 Apo-A1	0	4
HDL-1 Apo-A2	0	18.4
HDL-2 Apo-A2	0	6.2
HDL-3 Apo-A2	0	14
HDL-4 Apo-A2	0	2.5

LDL, low-density lipoprotein; HDL, high-density lipoprotein; Apo-A1, apolipoprotein A1; Apo-A2, apolipoprotein A2; Apo-B, apolipoprotein B; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein.

Appendix 7. .

The estimated regression coefficient from the least absolute shrinkage and selection operator method with percentage of models from the 1000 bootstrap samples that include each of the lipoprotein subfractions and CVD risk factors.

Lipid variables and CVD risk factors	Estimated regression coefficient	Percent included
Age	0	49.7
Hyperkolesterol	0	53.2
Hypertension, medically treated	−49.497	91.7
Diabetes mellitus	0	43
Previous CVD	0	66
Hereditary CVD	0	61.9

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Lipid variables and CVD risk factors	Estimated regression coefficient	Percent included
Body mass index	0	39.3
Smoking	0	48.4
Total plasma Triglycerides	0	0
Total plasma Cholesterol	0	2.5
LDL-C	0	1.3
HDL-C	0	3.7
Total Plasma Apo-A1	0	11.6
Total Plasma Apo-A2	0	5.3
Total Plasma Apo-B	0	0.3
Apo-B/Apo-A1	0	2.1
LDL-C/HDL-C	0	1.2
Lipoprotein a	0	67.1
Total Plasma Particle Number	0	0.3
VLDL Particle Number	0	0.4
IDL Particle Number	0	2.8
LDL Particle Number	0	0
LDL-1 Particle Number	0	7
LDL-2 Particle Number	0	5.8
LDL-3 Particle Number	0	1.2
LDL-4 Particle Number	0	1.7
LDL-5 Particle Number	0	0.1
LDL-6 Particle Number	0	7.4
VLDL Triglycerides	0	0
IDL Triglycerides	0	5.1
LDL Triglycerides	0	19.8
HDL Triglycerides	0	25
VLDL Cholesterol	0	0.7
IDL Cholesterol	0	3.7
VLDL Free Cholesterol	0	0
IDL Free Cholesterol	0	2.5
LDL Free Cholesterol	0	0.8
HDL Free Cholesterol	0	14.6
VLDL Phospholipids	0	1.6
IDL Phospholipids	0	1.2
LDL Phospholipids	0	0.1
HDL Phospholipids	0	0.9
HDL Apo-A1	0	5
HDL Apo-A2	0	5.7
VLDL Apo-B	0	0.7
IDL Apo-B	0	1.2
LDL Apo-B	0	0
VLDL-1 Triglycerides	0	6.3
VLDL-2 Triglycerides	0	1.9
VLDL-3 Triglycerides	0	1.6
VLDL-4 Triglycerides	0	7.4
VLDL-5 Triglycerides	0	16.3
VLDL-1 Cholesterol	0	1.5
VLDL-2 Cholesterol	0	4
VLDL-3 Cholesterol	0	5
VLDL-4 Cholesterol	0	6.2
VLDL-5 Cholesterol	0	3.5
VLDL-1 Free Cholesterol	0	1.9
VLDL-2 Free Cholesterol	0	2.5
VLDL-3 Free Cholesterol	0	0.1
VLDL-4 Free Cholesterol	0	4.6
VLDL-5 Free Cholesterol	0	25.9
VLDL-1 Phospholipids	0	7.2
VLDL-2 Phospholipids	0	0.6
VLDL-3 Phospholipids	0	5.9
VLDL-4 Phospholipids	0	4.4
VLDL-5 Phospholipids	0	44.8
LDL-1 Triglycerides	0	2.4
LDL-2 Triglycerides	0	9.4
LDL-3 Triglycerides	0	8.5
LDL-4 Triglycerides	0	18.6
LDL-5 Triglycerides	0	5.7
LDL-6 Triglycerides	0	26.1
LDL-1 Cholesterol	0	23.4
LDL-2 Cholesterol	0	8.5
LDL-3 Cholesterol	0	2.4
LDL-4 Cholesterol	0	1.3
LDL-5 Cholesterol	0	2.3
LDL-6 Cholesterol	0	1.2
LDL-1 Free Cholesterol	0	2.9
LDL-2 Free Cholesterol	0	16.3
LDL-3 Free Cholesterol	0	4

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Lipid variables and CVD risk factors	Estimated regression coefficient	Percent included
LDL-4 Free Cholesterol	0	1.2
LDL-5 Free Cholesterol	0	5.8
LDL-6 Free Cholesterol	0	55.4
LDL-1 Phospholipids	0	3
LDL-2 Phospholipids	0	9.8
LDL-3 Phospholipids	0	12.7
LDL-4 Phospholipids	0	21.3
LDL-5 Phospholipids	0	2.1
LDL-6 Phospholipids	0	0.2
LDL-1 Apo-B	0	4.4
LDL-2 Apo-B	0	5.7
LDL-3 Apo-B	0	0.9
LDL-4 Apo-B	0	1.6
LDL-5 Apo-B	0	0.1
LDL-6 Apo-B	0	7.2
HDL-1 Triglycerides	0	26.9
HDL-2 Triglycerides	0	6
HDL-3 Triglycerides	0	1.7
HDL-4 Triglycerides	0	28.2
HDL-1 Cholesterol	0	17.8
HDL-2 Cholesterol	0	4.6
HDL-3 Cholesterol	0	32.2
HDL-4 Cholesterol	0	6.7
HDL-1 Free Cholesterol	0	8.9
HDL-2 Free Cholesterol	0	28.6
HDL-3 Free Cholesterol	0	14.7
HDL-4 Free Cholesterol	0	44.6
HDL-1 Phospholipids	0	5.6
HDL-2 Phospholipids	0	2.9
HDL-3 Phospholipids	0	27.7
HDL-4 Phospholipids	0	2.9
HDL-1 Apo-A1	0	1.5
HDL-2 Apo-A1	0	1.2
HDL-3 Apo-A1	0	4.2
HDL-4 Apo-A1	0	3
HDL-1 Apo-A2	0	23.8
HDL-2 Apo-A2	0	4.6
HDL-3 Apo-A2	0	9.7
HDL-4 Apo-A2	0	2.3

CVD, cardiovascular disease; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; Apo-A1, apolipoprotein A1; Apo-A2, apolipoprotein A2; Apo-B, apolipoprotein B; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein.

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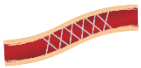
Patient inclusion

N = 56
Stable CAD

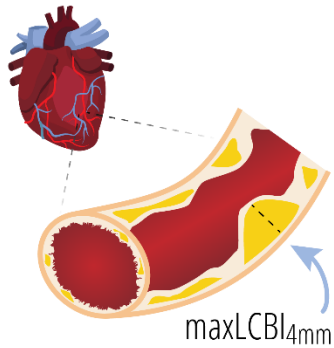


PCI
Drug-eluting stent

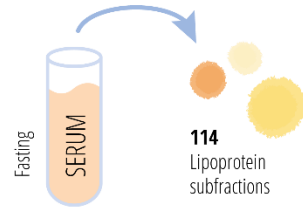
Statin therapy

Bypass graft, inflammatory diseases

Intracoronary NIRS



NMR Spectroscopy & CVD risk factors

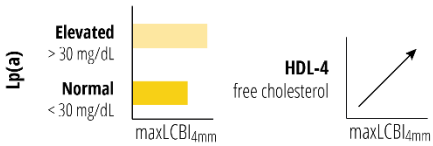


- Conventional lipid measures
- Hypertension
- Hereditary CVD
- Previous CVD
- Diabetes
- Smoking
- Age
- Sex
- BMI

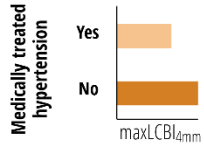
Results

Potential predictors of coronary lipid content (maxLCBI$_{4mm}$):

1 Lipoprotein subfractions only



2 Lipoprotein subfractions & CVD risk factors



CRITERIA Lasso regression coefficient ≠ 0 and ≥ 70% presence in bootstrap samples

Conclusion

Lp(a) and free cholesterol in the smallest HDL subfractions, HDL-4, were the lipoprotein subfractions with the strongest potential as predictors of coronary lipid content in statin-treated patients with stable CAD.

Although the evidence is modest, our study suggests that measurement of lipoprotein subfractions may provide additional information with respect to coronary plaque composition compared to traditional lipid measurements, but not in addition to other established risk factors.

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